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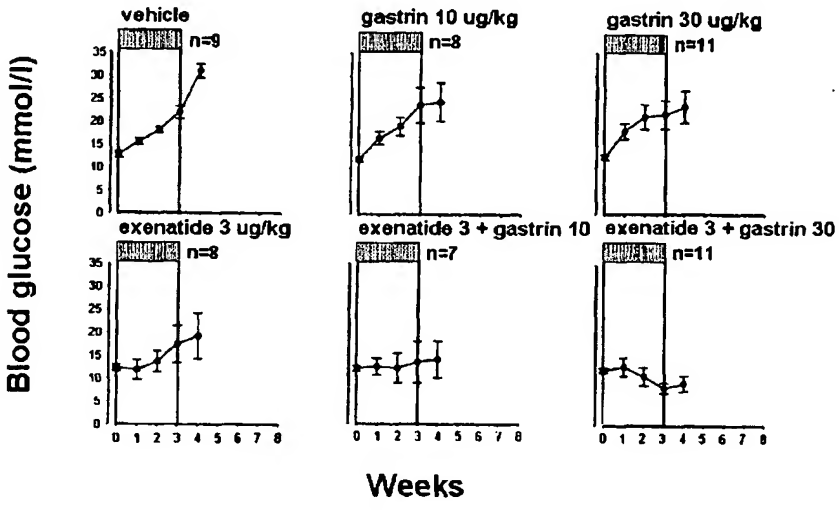
(54) Title: COMBINATION THERAPY FOR THE TREATMENT OF DIABETES COMPRISING AN EXENDIN AGONIST AND A GASTRIN COMPOUND



(57) Abstract: The invention relates to compositions, conjugates, and methods for the prevention and/or treatment of a condition and/or disease comprising a therapeutically effective amount of an exendin agonist and a gastrin compound. The combination of an exendin agonist and a gastrin compound provides beneficial effects, in particular sustained beneficial effects, in the prevention and/or treatment of conditions and/or diseases for which either an exendin agonist or a gastrin compound have been demonstrated to have a therapeutic effect, including but not limited to diabetes, hypertension, chronic heart failure, fluid retentive states, obesity, metabolic syndrome and related diseases and disorders. Combinations of an exendin agonist and a gastrin compound can be selected to provide unexpectedly additive effects or synergistic effects.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

COMBINATION THERAPY FOR THE TREATMENT OF DIABETES COMPRISING AN EXENDIN AGONIST AND A GASTRIN COMPOUND

FIELD OF THE INVENTION

The invention relates generally to compositions, conjugates, and methods comprising an exendin agonist and a gastrin compound, and uses thereof.

BACKGROUND OF THE INVENTION

Glucoregulatory peptides, such as incretins, are currently being used and investigated as potential therapies for Type 2 diabetes. Exenatide (exendin-4) is a 39-amino acid peptide incretin mimetic that exhibits activities similar to the mammalian incretin hormone glucagon-like peptide 1 (GLP-1). Exenatide is used in combination with metformin and/or a sulfonylurea medication to treat Type 2 diabetes. It has been reported that exenatide decreases blood glucose toward target levels and is associated with weight loss. The effects on glucose control are thought to be due to several properties including stimulating the insulin response in response to glucose and preventing glucagon (a hormone which raises blood sugar) release after meals.

SUMMARY OF THE INVENTION

The combination of an exendin agonist and a gastrin compound provides beneficial effects in the prevention and/or treatment of conditions and/or diseases for which exendin agonists or gastrin compounds have been demonstrated to have a therapeutic effect, including but not limited to diabetes, hypertension, chronic heart failure, fluid retentive states, obesity, metabolic syndrome and related conditions. Combinations of an exendin agonist and a gastrin compound may be selected to provide unexpectedly additive effects or greater than additive effects i.e. synergistic effects.

A composition, conjugate or method comprising an exendin agonist and a gastrin compound employing different mechanisms to achieve maximum therapeutic efficacy, may improve tolerance to the therapy with a reduced risk of side effects that may result from higher doses or longer term monotherapies (i.e. therapies with each compound alone). A composition, conjugate, or method of the invention may permit the use of lower doses of an exendin agonist or gastrin compound with reduced adverse toxic effects of each compound. A suboptimal dosage may provide an increased margin of safety, and may also reduce the cost of a drug necessary to achieve prophylaxis and therapy. In certain aspects of the invention, the increased convenience of a single combination dosage unit may result in enhanced compliance. Other advantages of a composition, conjugate, or combination therapy may include longer duration of action, and/or longer duration of action or effectiveness at particularly low doses.

Broadly stated, the invention relates to compositions, conjugates, and methods for the prevention and/or treatment of a condition and/or disease disclosed herein comprising or consisting essentially of a therapeutically effective amount of an exendin agonist and a gastrin compound that provide beneficial effects.

A composition, conjugate, or method of the invention may provide sustained beneficial effects following treatment or termination of treatment. Prolonged efficacy may be evidenced in Type 1 and Type 2 diabetes by stimulation of β-cell regeneration, increases in pancreatic β cell mass, prolonged increases in C-

peptide production, increases in pancreatic insulin production, increases in pancreatic insulin content and insulin release into plasma, and/or about normal blood glucose levels compared with exendin alone.

In an aspect, the invention contemplates a composition, preferably a pharmaceutical composition, comprising or consisting essentially of an exendin agonist and a gastrin compound that provide beneficial effects relative to an exendin agonist alone. In another aspect the invention provides a pharmaceutical composition comprising an exendin agonist and a gastrin compound that provide beneficial effects, preferably sustained beneficial effects, following treatment. A pharmaceutical composition may optionally comprise a pharmaceutically acceptable carrier, excipient, or vehicle.

The invention also contemplates a pharmaceutical composition comprising an exendin agonist and a gastrin compound, both optionally together with pharmaceutically acceptable carriers, excipients, or vehicles in separate containers and intended for simultaneous or sequential administration to provide beneficial effects, preferably sustained beneficial effects.

The invention further contemplates a conjugate comprising an exendin agonist interacting with or linked to a gastrin compound to provide beneficial effects, preferably sustained beneficial effects discussed herein.

The invention still further contemplates methods for preparing compositions and conjugates of the invention that result in compositions and conjugates with beneficial effects, preferably sustained beneficial effects.

In an aspect of the invention, a method is provided for preparing a pharmaceutical composition of an exendin agonist and a gastrin compound adapted to provide beneficial effects, preferably sustained beneficial effects, following treatment, comprising preparing a composition comprising the exendin agonist, a gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle. In a particular aspect of the invention, a method is provided for preparing a stable pharmaceutical composition of an exendin agonist and a gastrin compound adapted to provide beneficial effects, preferably sustained beneficial effects, following treatment, comprising preparing a composition comprising the exendin agonist, a gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle effective to physically stabilize the exendin agonist. In another aspect of the invention, a method is provided for preparing a stable pharmaceutical composition of an exendin agonist comprising mixing an exendin agonist, a gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle effective to physically stabilize the exendin agonist and adapted to provide beneficial effects, preferably sustained beneficial effects.

The invention relates to a combination treatment for preventing and/or treating a condition and/or disease disclosed herein in a subject comprising administering to the subject a therapeutically effective amount of at least one exendin agonist and a gastrin compound to provide beneficial effects. In an aspect the invention provides a combination treatment or intervention which provides sustained beneficial effects following treatment.

The invention further relates to the use of an exendin agonist and a gastrin compound, a composition, or conjugate of the invention for preventing, and/or ameliorating disease severity, disease symptoms, and/or

periodicity of recurrence of a condition and/or disease disclosed herein. The invention still further relates to the prevention and/or treatment, in a subject, of diseases and/or conditions disclosed herein using an exendin agonist and a gastrin compound, a composition, or conjugate of the invention.

In an aspect, the invention provides a method for the prevention and/or intervention of a condition and/or disease disclosed herein in a subject comprising administration of at least one exendin agonist and at least one gastrin compound, or a composition or conjugate of the invention. An exendin agonist and a gastrin compound, composition or conjugate may be directly administered to a subject or contacted with cells (e.g. stem cells or progenitor cells) and administered to a subject.

In other aspects, the invention provides a method for the prevention and/or intervention of a condition and/or disease disclosed herein in a subject comprising administration of at least one exendin agonist and at least one gastrin compound to a subject in need thereof to provide beneficial effects.

In another aspect, the invention provides a method for the prevention and/or intervention of a condition and/or disease discussed herein in a subject comprising co-administering at least one exendin agonist and at least one gastrin compound to a subject in need thereof.

In a particular aspect, the invention relates to inducing islet neogenesis in a subject comprising contacting pancreatic islet precursor cells with an exendin agonist and a gastrin compound, composition, or conjugate of the invention in a sufficient amount to increase proliferation of pancreatic islet precursor cells in the subject thereby inducing islet neogenesis.

The invention provides in some aspects methods for the potentiation of an exendin agonist in the treatment of a condition and/or disease in a subject, in particular diabetes and related diseases, disorders, or conditions, comprising co-administering with the exendin agonist at least one gastrin compound to the subject.

The invention provides in some aspects methods for the potentiation of a gastrin compound in the treatment of a condition and/or disease in a subject, in particular diabetes and related diseases, disorders, or conditions, comprising co-administering with the gastrin compound at least one exendin agonist to the subject.

In another aspect, the invention provides a method for the prevention and/or intervention of a condition and/or disease disclosed herein in a subject comprising co-administering at least one exendin agonist and at least one gastrin compound to a subject in need thereof.

In another aspect, the invention relates to a method for treating diabetes mellitus in a patient in need thereof by administering a gastrin compound and an exendin agonist or a composition comprising a gastrin compound and an exendin agonist in an amount sufficient to effect differentiation of the patient's pancreatic islet precursor cells to mature insulin-secreting cells and/or to stimulate insulin synthesis in existing islet cells.

The invention provides methods for treating cells using an exendin agonist and gastrin compound of the invention, or compositions, or conjugates of the invention. In particular, the invention relates to a method for expanding and differentiating stem cells, progenitor cells or islet precursor cells into islet cells in particular insulin secreting cells, enhancing proliferation of insulin secreting cells, and/or sustaining islet cells in particular

insulin secreting cells or islet precursor cells. Cells may be contacted with an exendin agonist and a gastrin compound in culture or in a subject.

In an aspect, a method is provided for treating a condition and/or disease disclosed herein comprising administering an exendin agonist and a gastrin compound, a composition or conjugate of the invention, with a plurality of cells to a subject in need thereof to thereby produce beneficial effects, preferably sustained beneficial effects. In an embodiment, the compounds, composition or conjugate are administered systemically.

In another aspect, the invention provides a method for treating a subject with a condition and/or disease disclosed herein comprising contacting *ex vivo* a plurality of cells with an exendin agonist and a gastrin compound, or a composition or conjugate of the invention, optionally culturing the cells, and administering the cells to the subject in need thereof.

Also provided in particular aspects of the invention are methods and compositions for treating diabetes in a patient in need thereof by implanting into a diabetic patient pancreatic islet cells that have been exposed in culture to a sufficient amount of a gastrin compound and an exendin agonist, or a composition or conjugate of the invention, to increase the number of pancreatic beta cells in the islets in the patient; optionally the population of pancreatic islet cells can be grown in culture for a time sufficient to expand a population of pancreatic beta cells prior to transplantation.

The invention also contemplates the use of a composition comprising a combination of at least one exendin agonist and at least one gastrin compound for the preparation of one or more medicament for preventing and/or treating a condition and/or disease. The invention further contemplates use of an exendin agonist in combination with a gastrin compound for the manufacture of a medicament for the treatment of a condition and/or disease. Still further the invention provides use of an exendin agonist for the manufacture of a medicament for the treatment of a condition and/or disease to be used in combination with a gastrin compound.

In an aspect, the invention relates to the use of synergistically effective amounts of at least one exendin agonist and at least one gastrin compound for the preparation of a medicament for preventing or treating a condition and/or disease disclosed herein. In another aspect, the invention relates to the use of an exendin agonist and a gastrin compound for the preparation of a medicament which has a protracted profile of action relative to an exendin agonist alone. The invention additionally provides uses of a pharmaceutical composition and a conjugate of the invention in the preparation of medicaments for the prevention and/or treatment of conditions and/or diseases disclosed herein. The medicaments provide beneficial effects, preferably sustained beneficial effects following treatment. Medicaments may comprise an exendin compound and a gastrin compound in separate containers and intended for simultaneous or sequential administration, in particular to provide beneficial effects, preferably sustained beneficial effects.

Since the present invention relates to a method of prevention and/or treatment comprising a combination of active agents which may be administered separately or as conjugates, the invention also provides a kit comprising an exendin agonist and a gastrin compound, separately or together, and a pharmaceutical composition or conjugate of the invention in kit form.

These and other aspects, features, and advantages of the present invention should be apparent to those skilled in the art from the following detailed description.

DESCRIPTION OF THE DRAWING

The invention will be better understood with reference to the drawings in which:

Figure 1 are graphs showing blood glucose levels (mMol/l) in NOD mice treated with vehicle, gastrin (10 μg/kg), gastrin (30 μg/kg), exenatide (3 μg/kg), exenatide (3 μg/kg) + gastrin (10 μg/kg), and exenatide (3 μg/kg) + gastrin (30 μg/kg).

Figure 2 are graphs showing blood glucose levels (mMol/l) in NOD mice treated with vehicle, gastrin (10 μg/kg), gastrin (30 μg/kg), exenatide (3 μg/kg), exenatide (3 μg/kg) + gastrin (10 μg/kg), and exenatide (3 μg/kg) + gastrin (30 μg/kg).

Figure 3 is a bar graph showing blood glucose levels (mMol/l) in NOD before therapy, NOD diabetic, NOD normoglycemic, and NOD-scid, treated with vehicle, gastrin (10 μg/kg), gastrin (30 μg/kg), exenatide (3 μg/kg), exenatide (3 μg/kg) + gastrin (10 μg/kg), and exenatide (3 μg/kg) + gastrin (30 μg/kg).

Figure 4 is a bar graph showing HbA1c (%) in NOD before therapy, NOD diabetic, NOD normoglycemic, and NOD-scid, treated with vehicle, gastrin (10 μg/kg), gastrin (30 μg/kg), exenatide (3 μg/kg), exenatide (3 μg/kg) + gastrin (10 μg/kg), and exenatide (3 μg/kg) + gastrin (30 μg/kg).

Figure 5 is a bar graph showing pancreatic insulin levels (μg) in NOD before therapy, NOD diabetic, NOD normoglycemic, and NOD-scid, treated with vehicle, gastrin (10 μg/kg), gastrin (30 μg/kg), exenatide (3 μg/kg), exenatide (3 μg/kg) + gastrin (10 μg/kg), and exenatide (3 μg/kg) + gastrin (30 μg/kg).

Figure 6 is bar graph showing plasma C-peptide levels (pmmol/ml) in NOD before therapy, NOD diabetic, NOD normoglycemic, and NOD-scid, treated with vehicle, gastrin (10 μg/kg), gastrin (30 μg/kg), exenatide (3 μg/kg), exenatide (3 μg/kg) + gastrin (10 μg/kg), and exenatide (3 μg/kg) + gastrin (30 μg/kg).

Figure 7 is a graph showing the correlation of blood glucose levels (mmol/l) and pancreatic insulin content (μg) in NOD mice treated with exenatide and gastrin.

Figure 8 is a bar graph showing fasting blood glucose levels (mM) in NOD mice treated with vehicle, GLP-1 (300 μg/kg/day), and gastrin (3 μg/kg/day) + GLP-1(300 μg/kg/day).

Figure 9 is a bar graph showing pancreatic insulin levels (μg/pancreas) in normal non-diabetic mice, NOD mice treated with vehicle, GLP-1, and gastrin + GLP-1.

Figure 10 shows histological examinations, in particular total β-cell mass, in NOD mice before treatment and after treatment with gastrin (3 μg/kg/day) + GLP-1(300 μg/kg/day).

Glossary

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, the terms "comprising," "including," and "such as" are used in their open and non-limiting sense.

The recitation of numerical ranges by endpoints herein includes all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term "about." The term "about" means plus or minus 0.1 to 50%, 5-50%, or 10-40%, preferably 10-20%, more preferably 10% or 15%, of the number to which reference is being made.

Further, it is to be understood that "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition or method comprising "a gastrin compound" includes a mixture of two or more gastrin compounds and a composition or method comprising "an exendin agonist" includes a mixture of two or more exendin agonists.

Selected compounds described herein may contain one or more asymmetric centers and may give rise to enantiomers, diasteriomers, and other stereoisomeric forms which may be defined in terms of absolute stereochemistry as (R)- or (S)-. Therefore, the invention includes all such possible diasteriomers and enantiomers as well as their racemic and optically pure forms. Optically active (R)- and (S)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and A geometric isomers. All tautomeric forms are intended to be included within the scope of the invention.

The terms "administering" and "administration" refer to the process by which a therapeutically effective amount of compounds or a composition or conjugate contemplated herein is delivered to a subject for prevention and/or treatment purposes. Compositions are administered in accordance with good medical practices taking into account the subject's clinical condition, the site and method of administration, dosage, patient age, sex, body weight, and other factors known to physicians.

The terms "subject", "individual" or "patient" refer to an animal including a warm-blooded animal such as a mammal, which is afflicted with or suspected of having or being pre-disposed to a condition and/or disease disclosed herein. Preferably, the terms refer to a human. The terms also include domestic animals bred for food, sport, or as pets, including horses, cows, sheep, poultry, fish, pigs, cats, dogs, and zoo animals. The methods herein for use on subjects/individuals/patients contemplate prophylactic as well as curative uses. Typical subjects for treatment include persons susceptible to, suffering from or that have suffered a condition and/or disease disclosed herein. A subject may or may not have a genetic predisposition for a condition and/or disease disclosed herein. In some aspects, a subject shows symptoms of a condition and/or disease.

The term "pharmaceutically acceptable carrier, excipient, or vehicle" refers to a medium which does not interfere with the effectiveness or activity of an active ingredient and which is not toxic to the hosts to which it is administered. A carrier, excipient, or vehicle includes diluents, binders, adhesives, lubricants, disintegrates, bulking agents, wetting or emulsifying agents, pH buffering agents, and miscellaneous materials such as absorbants that may be needed in order to prepare a particular composition. The use of such media and agents for

an active substance is well known in the art. In certain aspects of the invention, a carrier, excipient, or vehicle is selected to stabilize an exendin agonist and/or gastrin compound.

The compounds disclosed herein also include "pharmaceutically acceptable salt(s)". By pharmaceutically acceptable salts is meant those salts which are suitable for use in contact with the tissues of a subject or patient without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art and are described for example, in S. M. Berge, et al., J. Pharmaceutical Sciences, 1977, 66:1. Pharmaceutically acceptable salt(s) include acidic or basic groups which may be present in the compounds disclosed herein. Acids which are used to prepare pharmaceutically acceptable acid addition salts of compounds disclosed herein are those which form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulfonate, ethanesulfonate, benzenesulfonate, para-toluenesulfonate and pamoate [i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)] salts. Suitable non-toxic base salts include, without limitation, those derived from such pharmacologically acceptable cations such as alkali metal cations (e.g., potassium and sodium) and alkaline earth metal cations (e.g., calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine (meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines.

The terms "preventing and/or treating", "prevention and/or treatment", or "prevention and/or intervention" refer to the administration to a subject of biologically active agents either before or after onset of a condition and/or disease. A treatment may be either performed in an acute or chronic way. In particular, treatment or intervention refers to the management and care of a subject at diagnosis or later, and prevention includes the management and care of a subject at risk of developing a condition and/or disease prior to the clinical onset of the condition and/or disease. The terms also include preventing or reducing the severity of a condition and/or disease or symptoms associated with such condition and/or disease prior to affliction with the disease. The terms also refer to preventing the recurrence of a condition and/or disease or of one or more symptoms associated with such condition and/or disease. An objective of prevention, treatment, or intervention is to combat the condition and/or disease and includes administration of the active compounds to prevent or delay the onset of the symptoms or complications, or alleviating the symptoms or complications, or eliminating or partially eliminating the condition and/or disease.

A "beneficial effect" refers to an effect of a combination of an exendin agonist and a gastrin compound, or composition or conjugate thereof that is greater than the effect of either of the compounds alone. The beneficial effect includes favourable pharmacological effects, therapeutic effects, and/or improved pharmacokinetic properties and/or biological activity. A beneficial effect may be an additive effect or synergistic effect. In embodiments of the invention related to Type 1 or Type 2 diabetes, beneficial effects include but are not limited to the following: reduced or absent islet inflammation, stimulation of β-cell regeneration, effective β-

cell replacement, normalizing hyperglycemia, preventing, slowing or reducing development of hyperglycemia, increased pancreatic β-cell mass, increasing β-cell mass in pancreatic ducts, increased pancreatic insulin content, increased release of insulin into plasma, decreased disease progression, increased survival, or elimination or partial elimination of a condition and/or disease. In particular embodiments, the beneficial effect is a "sustained beneficial effect" where the beneficial effect is sustained for a prolonged period of time after termination of treatment. In an embodiment, one or more of the aforementioned effects are sustained for a prolonged period of time after termination of treatment. A beneficial effect may be sustained for at least about 1, 2, 4, 6, 8, 10, 1 to 4 weeks, 2 to 4 weeks, 2 to 6 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment. The period of time a beneficial effect is sustained may correlate with the duration and timing of the treatment. A subject may be treated continuously for about 2 to 8 weeks, 2 to 12 weeks, 2 to 16 weeks, 2 weeks to 6 months, 2 weeks to 12 months, or periodically. A sustained beneficial effect may manifest as one or more of stimulation of β-cell regeneration, increased C-peptide production, increased pancreatic insulin production, increased pancreatic insulin content, increased pancreatic β-cell mass, increased release of insulin into plasma, and/or, about normal or low blood glucose levels for a prolonged period following treatment. The beneficial effect may be a statistically significant effect in terms of statistical analysis of an effect of the two compounds versus the effects of each of the compounds. "Statistically significant" or "significantly different" effects or levels with two compounds compared with each compound alone may represent levels that are higher or lower than a standard. In embodiments of the invention, the difference may be 1.5, 2, 3, 4, 5, or 6 times higher or lower compared with the effect obtained with each compound alone.

An "additive effect" of an exendin agonist and a gastrin compound refers to an effect that is equal to the sum of the effects of the two individual compounds

A "synergistic effect" of an exendin agonist and a gastrin compound refers to an effect that is greater than the additive effect which results from the sum of the effects of the two individual compounds.

"Combination treatment", "combination therapy", and "administering in combination" are used interchangeably herein and mean that the active ingredients are administered concurrently to a patient being treated. When administered in combination each component may be administered at the same time, or sequentially in any order at different points in time. Therefore, each component may be administered separately, but sufficiently close in time to provide the desired effect, in particular a beneficial, additive, or synergistic effect. The first compound may be administered in a regimen which additionally comprises treatment with the second compound. In certain embodiments, the term refers to administration of an exendin agonist and a gastrin compound to a patient within one week, two weeks, three weeks, one, two three, four, five or six months, or one year, including separate administration of two medicaments each containing one of the compounds as well as simultaneous administration whether or not the two compounds are combined in one formulation or whether they are two separate formulations.

A "medicament" refers to a pharmaceutical composition suitable for administration of a pharmaceutically active compound(s) (e.g. an exendin agonist and/or a gastrin compound) to a patient.

"Therapeutically effective amount" relates to the amount or dose of active compounds (e.g. exendin agonist and gastrin compound), compositions or conjugates of the invention that will lead to one or more desired beneficial effects, preferably one or more sustained beneficial effects. A "therapeutically effective amount" can provide a dosage which is sufficient in order for prevention and/or treatment of a subject to be effective compared with no treatment. A therapeutically effective amount of a substance can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance to elicit a desired response in the individual. A dosage regimen may be adjusted to provide the optimum therapeutic response (e.g. one or more beneficial effect, in particular a sustained beneficial effect). For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

"Synergistically effective amount" relates to the amount of dose of active compounds (e.g. exendin agonist and gastrin compound), compositions or conjugates of the invention that will provide a synergistic effect, in particular a synergistic beneficial effect.

The expression "complement" or "complementary activity" refers to the pharmacological action of one or two or more different compounds making it possible to act on the same pathology via different pharmacological mechanisms, for example the combined use of an exendin agonist and a gastrin compound.

The term "potentiation" refers to an increase of a corresponding pharmacological activity or therapeutic effect. Potentiation of one component of a combination or composition of the present invention by co-administration of the other components according to the present invention means that an effect is being achieved that is greater than that achieved with one component alone.

"Suboptimal dose" or suboptimal dosage" refers to a dose or dosage of an active compound which is less than the optimal dose or dosage for that compound when used in mono-therapy.

The terms "associated", "linked", "interact", "interaction", or "interacting" refer to any physical association between molecules. The terms preferably refer to a stable association between two molecules due to, for example, electrostatic, hydrophobic, ionic, hydrogen-bond interactions, or covalent interactions. Certain interacting or associated molecules interact only after one or more of them have been activated.

"Sequence identity" of two amino acid sequences, or of two nucleic acid sequences is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues in a polypeptide or nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining amino acid or nucleic acid sequence identity can be achieved in various conventional ways, for instance, using publicly available computer software including the GCG program package (Devereux J. et al., Nucleic Acids Research 12(1): 387, 1984); BLASTP, BLASTN, and FASTA (Atschul, S.F. et al. J. Molec. Biol. 215: 403-410, 1990). The BLAST programs are publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al. NCBI NLM NIH Bethesda, Md. 20894; Altschul, S. et al. J. Mol. Biol. 215: 403-410, 1990). Skilled artisans can determine appropriate

parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Methods to determine identity and similarity are codified in publicly available computer programs.

An "analog" refers to a polypeptide wherein one or more amino acid residues of a parent or wild-type polypeptide have been substituted by another amino acid residue, one or more amino acid residues of a parent or wild-type polypeptide have been inverted, one or more amino acid residues of the parent or wild-type polypeptide have been deleted, and/or one or more amino acid residues have been added to the parent or wild-type polypeptide. Such an addition, substitution, deletion, and/or inversion may be at either of the N-terminal or C-terminal end or within the parent or wild-type polypeptide, or a combination thereof. Typically "an analog" is a peptide wherein 6 or less amino acids have been substituted and/or added and/or deleted from the parent or wild-type peptide, more preferably a peptide wherein 3 or less amino acids have been substituted and/or added and/or deleted from the parent or wild-type polypeptide, and most preferably, a peptide wherein one amino acid has been substituted and/or added and/or deleted from the parent or wild-type polypeptide.

Mutations may be introduced into a polypeptide by standard methods, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative substitutions can be made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which an amino acid residue is replaced with an amino acid residue with a similar side chain. Amino acids with similar side chains are known in the art and include amino acids with basic side chains (e.g. Lys, Arg, His), acidic side chains (e.g. Asp, Glu), uncharged polar side chains (e.g. Gly, Asp, Glu, Ser, Thr, Tyr and Cys), nonpolar side chains (e.g. Ala, Val, Leu, Iso, Pro, Trp), beta-branched side chains (e.g. Thr, Val, Iso), and aromatic side chains (e.g. Tyr, Phe, Trp, His). Mutations can also be introduced randomly along part or all of the native sequence, for example, by saturation mutagenesis. Following mutagenesis the variant polypeptide can be recombinantly expressed.

A "derivative" refers to a polypeptide in which one or more of the amino acid residues of a parent polypeptide have been chemically modified. Derivatives may be obtained by chemically modifying one or more amino acid residues of the parent polypeptide or analog thereof, for instance by alkylation, acylation, glycosylation, pegylation, ester formation, deamidation, amide formation, or by introducing a lipophilic functionality. In aspects of the invention, "a derivative" designates a peptide or analogue thereof which is chemically modified by introducing an ester, alkyl or lipophilic functionality on one or more amino acid residues of the peptide or analogue thereof.

A "chimeric polypeptide" comprises all or part (preferably biologically active) of a selected polypeptide operably linked to a heterologous polypeptide (i.e., a polypeptide other than the selected polypeptide). Within the chimeric polypeptide, the term "operably linked" is intended to indicate that a selected polypeptide and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of a selected polypeptide. Chimeric and fusion proteins can be produced by standard recombinant DNA techniques.

An "exendin agonist" includes naturally occurring exendin peptides that are found in the salivary secretions of the Gila-monster and the Mexican Bearded Lizard, reptiles that are endogenous to Arizona and Northern Mexico. Exendin-3 [SEQ. ID. NO. 10) is present in the salivary secretions of *Heloderma horridum* (Mexican Beaded Lizard), and exendin-4 (SEQ. ID. NO. 11) is present in the salivary secretions of *Heloderm suspectum* (Gila monster) (Eng, J., et al., *J. Biol. Chem.*, 265:20259-62, 1990; Eng, J., et al., *J. Biol. Chem.*, 267:7402-05, 1992). An "exendin agonist" also includes analogues and derivatives of a naturally occurring exendin peptide, in particular a synthetic analogue or derivative or a stable exendin analogue or derivative of a naturally occurring exendin peptide.

In embodiments of the invention, the exendin agonist is an exendin-3. In other embodiments, the exendin is exendin-4. In particular embodiments, the exendin is exenatide, more particularly an injectable exenatide or long-acting release exenatide, most particularly Byetta® or long-acting release Byetta®.

Exendin analogues also include functional variants having similar amino acid sequences and retaining, to some extent, the activities of the related exendin. By "functional variant" is meant an analog which has an activity that can be substituted for one or more activities of a particular exendin. Preferred functional variants retain all of the activities of a particular exendin, however, the functional variant may have an activity that, when measured quantitatively, is stronger or weaker, as measured in exendin functional assays, for example, such as those disclosed in US6924264.

A "stable exendin analogue or derivative" means an exendin analogue or a derivative which exhibits an *in vivo* plasma elimination half-life of at least 10 hours in a subject as determined by the method described below.

Examples of exendin analogues and derivatives include an exendin-4(1-39) analogue or a derivative of an exendin-4(1-39).

In aspects, an exendin agonist has the sequence of SEQ. ID NO. 10, 11, 12, 13 or 14, or an analogue or derivative thereof.

Examples of exendin agonists as well as analogues, derivatives, and fragments thereof to be included within the present invention are those disclosed in WO 97/46584, US 5,424,286 and WO 01/04156. US 5,424,286 discloses exendin polypeptides including HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGX [SEQ. ID NO. 1]; wherein X = P or Y, and HX1X2GTFITSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS [SEQ. ID NO. 2]; wherein X1X2 = SD (exendin-3) or GE (exendin-4)). WO 97/46584 discloses truncated versions of exendin peptide(s) that increase secretion and biosynthesis of insulin, but reduce those of glucagon. WO 01/04156 describes exendin-4 analogues and derivatives as well as the preparation of these molecules. Exendin-4 analogues stabilized by fusion to serum albumin or Fc portion of an Ig are disclosed in WO 02/46227.

In embodiments, an exendin agonist has the empirical formula $C_{184}H_{282}N_{50}O_{60}S$ and a molecular weight of 41.86.6 daltons, and the following amino acid sequence: H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2 [SEQ. ID NO. 14]. In an embodiment, the exendin agonist has an amino acid sequence

comprising His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser.

Particular exendin agonists include the following: exendin-4 (1-30) [SEQ. ID NO 4: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly]; exendin-4 (1-30) amide [SEQ. ID NO 5: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-NH_2]; exendin-4 (1-28) amide [SEQ. ID NO 6: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH_2]; ^{14}Leu, ^{25}Phe exendin-4 amide [SEQ. ID NO 7: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH_2]; ^{14}Leu, ^{25}Phe exendin-4 (1-28) amide [SEQ. ID NO 8: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH_2]; ^{14}Leu, ^{22}Ala, ^{25}Phe exendin-4 (1-28) amide [SEQ. ID NO 9: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Ala Ile Glu Phe Leu Lys Asn-NH_2]; and His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Ser Lys Lys Lys Lys Lys Lys [SEQ. ID NO. 29].

In another particular aspect, the pharmaceutical composition comprises exendin-4. In other preferred aspects, the pharmaceutical composition comprises a peptide selected from: exendin-4 (1-30), exendin-4 (1-30) amide, exendin-4 (1-28) amide, ^{14}Leu, ^{25}Phe exendin-4 amide, ^{14}Leu, ^{25}Phe exendin-4 (1-28) amide, and ^{14}Leu, ^{22}Ala, ^{25}Phe exendin-4 (1-28) amide.

Examples of exendin analogs include without limitation exendin agonist compounds such as those described in US20060035836A1, US20050267034A1, US6989366, US6956026, and publications and patents and patent applications referenced therein.

In embodiments, the exendin-4 analogue is HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPS SGAPPSKKKKKK [SEQ. ID NO. 3].

A "gastrin/CCK receptor" refers to a member of the G-protein-coupled receptor family that displays a characteristic binding affinity for a cholecystokinin (CCK) including without limitation CCK-8, desulfated CCK-8, CCK-33, CCK-4, or gastrins including without limitation desulfated or sulfated gastrin-17, or pentagastrin, or other CCK or gastrin analogues or family members. Examples of CCK/gastrin receptor proteins are CCK_A and CCK_B/gastrin receptors, in particular a CCK_B/gastrin receptor.

A "gastrin compound" refers to any compound, including peptides and non-peptide compounds, which fully or partially associate with and/or activate a gastrin/CCK receptor, in particular a gastrin/CCK_B receptor. In aspects of the invention, a gastrin compound is selected that has a suitable IC_{50}, for example an IC_{50} of about ~ 0.7 nM at a gastrin/CCK_B receptor, as measured by methods known in the art (see Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023 describing *in vitro* cell growth assays, and receptor binding assays as described in Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin

et al (1995) J. Biol. Chem. 270: 5019-5023). A gastrin compound may also be selected based on other criteria such as activity, half-life etc.

The term "gastrin compound" encompasses compounds that in combination with an exendin agonist provide at least one beneficial effect. In other aspects of the invention a gastrin compound is selected to complement or enhance an exendin agonist such that when a gastrin compound in the same or adjacent tissue is present in the same individual, neogenesis of insulin-producing pancreatic islet cells is induced. In a further aspect the term includes any gastrin compound that demonstrates additive, synergistic, or complementary activity with an exendin agonist.

Gastrin compounds that may be used in the present invention include without limitation one or more of a gastrin or a gastrin agonist. A gastrin includes, without limitation, the various forms of gastrin, such as gastrin 71, gastrin 52, gastrin 34 (big gastrin), gastrin 17 (little gastrin), gastrin 14, gastrin 8 (mini gastrin), pentagastrin, tetragastrin, and fragments, analogs, and derivatives thereof. Sequences for gastrins including big gastrin-34 (Bonato et al, 1986, Life Science 39:959) and small gastrin-17 (Bentley et al (1966) Nature 209:583) are known in the art, and some are shown in SEQ. ID NOs. 15 to 23. In particular, sequences for gastrins include gastrin 71 of SEQ. ID NO. 19, gastrin 52 of SEQ. ID NO. 20, gastrin 34 (big gastrin) of SEQ. ID NO. 15 or 16, gastrin 17 (little gastrin) of SEQ. ID NO. 17 or 18, gastrin 14 of SEQ. ID NO. 21, and gastrin 6 of SEQ. ID NO. 22 or 23. Gastrin-34 is essentially an extension of an amino acid sequence at the N-terminal end of gastrin-17. Big gastrin is cleaved *in vivo* to release gastrin-17. Glp at the N-terminal end of a gastrin is pyroglutamate, which is a naturally cyclized form of glutamate. In various embodiments, where cysteine or lysine is added to a terminus of gastrin having a pyroglutamate, the pyroglutamate is replaced with a glutamate, or the pyroglutamate is deleted. A gastrin 34 or gastrin-17 may be used in the invention where there is a methionine or a leucine at position 15, as shown in SEQ. ID NOs: 15-18 herein. In some aspects, a gastrin, in particular a gastrin 34 or gastrin 17 may not include a pyroglutamate.

Examples of gastrins that may be used in the present invention include the compounds disclosed in U.S. Patent No. 6,288,301. In some applications of the invention, a gastrin may be selected that is a peptide or non-peptide agonist or partial agonist of the gastrin receptor such as A71378 (Lin et al., Am. J. Physiol. 258 (4 Pt 1): G648, 1990).

In some applications of the invention, a gastrin compound may be selected that is a gastrin/CCK receptor ligand including but not limited to cholecystokinin (CCK) such as CCK 58, CCK 33, CCK 22, CCK 12 and CCK 8, or a cholecystokinin agonist, and the like. A gastrin compound may also include substances that increase the secretion of endogenous gastrins, cholecystokinins or similarly active peptides from sites of tissue storage. Examples of these are gastrin agonists, and soya bean trypsin inhibitor which increases CCK stimulation.

A "gastrin compound" includes analogs, derivatives, fragments and modifications of a wild-type gastrin compound and chimeric polypeptides comprising a gastrin compound. In aspects of the invention a gastrin compound includes a polypeptide that shares substantial amino acid sequence identity with a mammalian gastrin

and possesses some or all of the biological activity of a mammalian gastrin. In certain aspects, a gastrin compound may be an active analog, fragment or other modification which, for example, share amino acid sequence with an endogenous mammalian gastrin, for example, share 60% sequence identity, or 70% sequence identity, or 80% sequence identity.

A "gastrin compound" includes a modified form of a gastrin, including but not limited to a modified form of gastrin 71 [SEQ. ID NO. 19], gastrin 52 [SEQ. ID NO. 20], gastrin 34 (big gastrin) [SEQ. ID NO. 15 or 16], gastrin 17 (little gastrin) [SEQ. ID NO. 17 or 18], gastrin 14 [SEQ. ID NO. 21], gastrin 8, gastrin 6 [SEQ. ID NO. 22 or 23], pentagastrin, and tetragastrin. A modified gastrin preferably comprises TrpMetAspPhe-NH_2 [SEQ. ID NO. 27] or TrpLeuAspPhe-NH_2 [SEQ. ID NO. 28].

In aspects of the invention a modified gastrin comprises at least amino acids 1-34, 18-34 or 29-34 of SEQ. ID NO. 15 or 16, or amino acids 1-17, 2-17, 12-17, or 14-17 of SEQ. ID NO. 17 or 18.

A gastrin compound used in aspects of the methods, compositions, and conjugates of the invention may comprise gastrin 17 and analogs and derivatives thereof. In particular aspects, the gastrin compound is synthetic human gastrin 1 having 17 amino acid residues with a Leu residue at amino acid position 15 [SEQ. ID NO. 18].

A gastrin compound used in the methods, compositions and conjugates of the invention may comprise gastrin 34 and analogs and derivatives thereof. In particular aspects, the gastrin compound is a synthetic human gastrin 34 with methionine or leucine at position 32 [SEQ ID NO. 15 or 16].

Modified gastrin compounds for use in the present invention comprise the modified gastrin compounds described in PCT/CA03/01778 and US Application Serial No. 10/728,082 incorporated in their entirety by reference.

In particular, a modified gastrin can be a gastrin derivative or analogue comprising a minimal sequence of 6 amino acids (from the C-terminal end) of a gastrin, in particular amino acid residues 1 to 34, 18 to 34 or 29-34 of SEQ ID NO: 15 or 16, or amino acid residues 1-17, 2-17, 12-17, or 14-17 of SEQ ID NO. 17 or 18, and comprising a reactive group capable of undergoing an addition reaction. Examples of reactive groups include without limitation thiols, alpha amino groups, epsilon amino groups, carboxyl groups or aromatic rings. A reactive group is generally capable of linking a gastrin sequence, directly or indirectly via a crosslinking agent and/or spacer region, to a carrier.

A reactive group may be introduced by adding or substituting an amino acid comprising a reactive group, for example by adding a cysteine or lysine. Therefore, a modified gastrin may comprise a gastrin sequence (e.g. gastrin-34 or gastrin 17) wherein at least one reactive amino acid (e.g. cysteine or lysine) is added or substituted. The addition of a reactive amino acid can be at a terminal region, in particular an N-terminal region.

A modified gastrin may also optionally comprise a spacer. A spacer can interact with a reactive group, for example, an amino acid comprising a reactive group. A spacer can be one or more amino acids, peptides, peptidomimetics, or small organic molecules. A spacer can comprise at least one amino acid, preferably at least two, three, four or five amino acids and in certain embodiments it is a sequence of several amino acids, including

without limitation alanine or glycine. A spacer can comprise alternating amino acids (e.g. glycine and/or alanine), non-alternating amino acids, a random sequence or a particular sequence. By way of example, a spacer can be synthesized as part of, or may be chemically attached to an amino acid of a gastrin sequence.

A modified gastrin may optionally comprise a cross-linking agent. A cross-linking agent may comprise a homobifunctional or heterobifunctional portion for interaction directly or indirectly with a gastrin, spacer and/or a reactive group. A cross-linking agent may interact with a gastrin sequence or a spacer, or it may be added to a reactive group at the end (in particular N-terminus) of a modified gastrin.

A cross-linking agent can be any agent that can link a gastrin sequence and a carrier directly or via a spacer. Examples of homobifunctional crosslinking agents include without limitation amino group directed homobifunctional cross-linking reagents such as bisimidates (e.g. methyl acetimidate-HCl), bifunctional aryl halides (e.g. 1,5-dichloro-2,4-dinitrobenzene), bifunctional acylating agents (e.g. diisocyanates), bifunctional sulfonyl halides (e.g. phenol-2,4-disulfonyl-chloride), bifunctional acylazides (e.g. tartryl diazide), dialdehydes (e.g. glutaraldehyde), and diketones (e.g. 2,5-hexanedione). Examples of heterobifunctional crosslinkers include amino and sulfhydryl group directed bifunctional reagents (e.g. N-succinimidyl-3-(2-pyridyldithio propionate, carboxyl and either sulfhydryl or amino group directed bifunctional reagents (e.g. p-nitrophenyl diazoacetate), and carbonyl and sulfhydryl group directed bifunctional reagents (e.g. 1-(aminooxy)-4-[3-nitro-2-pyridyl)dithio)]butane).

A modified gastrin can optionally comprise a carrier which may be a polymer. A carrier may be a polymer of amino acids (proteins), sugars (polysaccharides), nucleosides, synthetic polymers and mixtures thereof. A protein carrier may be a protein found in the circulatory system. Examples of protein carriers found in the circulatory system, in particular the human circulatory system, include without limitation plasma components such as serum, purified serum proteins such as albumin (in particular human serum albumin), transferrin, or an immunoglobulin, red blood cell proteins such as glycophorin A and AE-1, sugar binding proteins such as a lectin, inactivated enzymes, phosphate and sulphate binding proteins, and lipid binding proteins. Examples of other suitable polymeric carriers include without limitation cellulose and derivatives thereof, starch and derivatives thereof, heparin and derivatives thereof, and synthetic polymers such as polyethylene glycol (PEG) and dextran, and derivatives thereof. Carriers may be attached to a gastrin or spacer by way of reactive groups on, or introduced to, the carrier, gastrin, and/or spacer. For example, carriers can be covalently attached to reactive groups (such as thiol groups, alpha and epsilon amino groups, carboxyl groups or aromatic groups) on a gastrin or spacer which may be present or added by chemical modification of the gastrin or spacer.

In certain aspects of the invention, a modified gastrin can comprise a gastrin of SEQ ID NOS 15, 16, 17, or 18 and a carrier.

A group of modified gastrin compounds include compounds having an amino acid sequence comprising from the amino terminus $Z\text{-}Y_m\text{-}X_n\text{-}AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$, wherein AA_1 is Tyr or Phe, AA_2 is Gly, Ala, or Ser, AA_3 is Trp, Val, or Ile, AA_4 is Met or Leu, AA_5 is Asp or Glu, and AA_6 is Phe or Tyr and wherein AA_6 is optionally amidated; Z is a carrier, in particular a polymer and when the polymer is a protein Z is an amino acid

sequence; Y_m is an optional spacer region comprising m amino acid residues of a small neutral amino acid including but not limited to serine and alanine, and X is any consecutive portion of residues 1-28 (=n) of SEQ ID NO: 15 or 16 or 1-11 of SEQ ID. NO. 17 or 18, providing that the gastrin compound binds a gastrin/CCK_B receptor. Generally, m is 0 to about 20 residues. In an aspect Z is a protein, in particular a protein of the circulatory system, more particularly a serum protein, still more particularly albumin, most particularly human serum albumin.

In embodiments, X is one or more amino acid residues from position 18 to position 28 of SEQ ID NO: 15. Therefore, the gastrin compounds by virtue of the presence of X, can have any of the gastrin sequences from positions 18-28, 19-28, 20-28, 21-28, etc. The gastrin compound optionally contains an amino acid spacer (Y) of length m, and m is 0 to about 20 residues.

In embodiments, X is one or more amino acid residues from position 1 to 11 or 2 to 11 of SEQ ID NO: 17 or 18. Therefore, the gastrin compounds by virtue of the presence of X, can have any of the gastrin sequences from positions 2 to 11, 3 to 11, 4 to 11, 5 to 11, etc. The gastrin compound optionally contains an amino acid spacer (Y) of length m, and m is 0 to about 20 residues.

A gastrin compound includes a modified gastrin of the formula X_n-AA_1-AA_2-AA_3-AA_4-AA_5-AA_6, where there is no spacer (Y) and m is 0, which may further comprise a bifunctional cross-linking agent for interaction or linkage to a carrier Z, where Z further comprises a non-proteinaceous polymer such as dextran or PEG.

A modified gastrin compound particularly described herein may further comprise an amino terminal cysteine or lysine residue.

In some embodiments of modified gastrin compounds described herein, the gastrin component contains at least amino acid residues 29-34 of SEQ ID NO: 15 or 16, and it is associated with a polymer, a lipid or a carbohydrate. The polymer may be a synthetic or naturally occurring polymer. The term polymer includes a protein polymer of amino acids, and is not limited to a synthetic polymer. The polymer may be a polyethylene glycol (PEG) or a dextran. A modified gastrin compound can be based on SEQ ID NO: 15 or 2 or "big" gastrin-34 and have a residue at position 32 which is a methionine or a leucine, respectively.

Another particular modified gastrin compound comprises a structure C-Y_m-X, wherein C is Cys or Lys, Y_m is an optional spacer region comprising m amino acid residues of a small neutral amino acid, and X is at least six amino acid residues comprising at least positions 12-17 of gastrin-17 (SEQ ID NO: 17 or 18) or at least positions 29-34 of gastrin-34 (SEQ ID NO: 15 or 16). This modified gastrin compound can further comprise a bifunctional cross-linking agent wherein one reactive portion of the cross-linking agent is covalently linked to C, and the other reactive portion is covalently linked to a polymer or protein.

In a particular aspect of the invention AA_1-AA_2-AA_3-AA_4-AA_5-AA_6 in a modified gastrin compound is Tyr-Gly-Trp-Met-Asp-Phe [SEQ ID NO. 24] or Tyr-Gly-Trp-Leu-Asp-Phe [SEQ ID NO. 25].

In a further aspect of the invention, a gastrin compound used in the methods, compositions and conjugates of the invention is gastrin 34 or gastrin 17 or portions thereof, directly or indirectly interacting or

associated with a serum protein, in particular albumin or an immunoglobulin, more particularly human serum albumin.

In aspects of the invention, a gastrin compound comprises synthetic human gastrin 34 having 2-34 amino acid residues of SEQ ID NO. 15 or 16, and optionally an N-terminal cysteine and/or a carrier; synthetic human gastrin having 1-17 amino acid residues with a Leu residue at amino acid position 15 [SEQ ID NO. 18] and optionally an N-terminal cysteine residue; and a synthetic human gastrin having amino acid residues 2 to 17 or 5-17 of SEQ ID NO. 17 or 18, optionally with an N-terminal cysteine residue and/or a carrier (e.g. PEG or human serum albumin) linked via a spacer [e.g. Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala i.e. $(GA)_5$] [SEQ ID NO. 26], in particular, a synthetic human gastrin having amino acid residues 2 to 17 or 5-17 of SEQ ID NO. 17 or 18, with a human serum albumin (HSA) polymer linked via a Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala [ie. $(GA)_5$] spacer, and optionally an N-terminal cysteine residue.

In particular aspects of the invention the gastrin compound is a leucine substituted gastrin 17 of SEQ ID NO. 18. Such a gastrin compound may also be characterized by the following properties: isoelectric point of about 3.4; purity of at least about 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and/or a molecular mass of about 2080.2 ±2 Da.

Gastrin compounds may be synthesized by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogenous solution (Houbenweyl, 1987, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart). The synthesis may be performed using manual procedures or by automation. Automated synthesis may be carried out, for example, using an Applied Biosystems 431A peptide synthesizer (Perkin Elmer). Gastrin compounds may also be obtained from commercial sources. For example, synthetic human gastrin 17 with methionine or leucine at position 15 are available from Bachem AG, Bubendorf, (Switzerland) and from Research Plus Inc (New Jersey, USA).

A "gastrin agonist" refers to any substance that fully or partially mimics a reaction, activity, or function of a gastrin or initiates such reaction, activity, or function, or reduces or prevents inhibition of any reaction, activity or function of a gastrin. A gastrin agonist can include substances that increase the secretion of endogenous gastrins, cholecystokinins or similarly active peptides from sites of tissue storage. In aspects of the invention, a gastrin agonist is a gastrin secretagogue. In aspects of the invention, a gastrin agonist is selected that provides, in combination with an exendin agonist beneficial effects in a subject (e.g., a diabetic subject). In particular aspects of the invention a gastrin agonist is selected that provides an about 1.5 to 1000 fold, 5 to 1000 fold, 10 to 1000 fold, 10 to 500 fold, 10 to 100 fold, 5 to 100, 10 to 50, 5 to 50, 10 to 25, 1.5 to 10, 1.5 to 5, 1.5 to 3, 1.5 to 5, 1.5 to 10, 1.5 to 20, 1.5 to 25, 3 to 5, 3 to 10, 3 to 15, 3 to 25, 5 to 15, or 5 to 20 fold increase in plasma gastrin. The term includes analogs, derivatives, fragments and modifications of a wild-type gastrin agonist and chimeric polypeptides comprising a gastrin agonist. Generally, a gastrin agonist is a proton pump inhibitor or a histamine-2 receptor antagonist.

A "proton pump inhibitor" and "PPI" are used interchangeably herein and include a substance which inhibits gastric acid secretion by blocking the proton pump and/or increasing gastrin secretion.

In particular, the term "proton pump inhibitor" refers to any acid labile pharmaceutical agent possessing pharmacological activity as an inhibitor of H^+/K^+-ATPase. More particularly it contemplates substances which covalently bind to H+/K+-ATPase, the enzyme responsible for gastric acid secretion. [See the following publications relating to PPIs: Fellenius et al., Substituted Benzimidazoles Inhibit Gastric Acid Secretion by Blocking H^+, K^+-ATPase, Nature, 290:159-161 (1981); Wallmark et al, The Relationship Between Gastric Acid Secretion and Gastric H^+, K^+-ATPase Activity, J. Biol.Chem., 260:13681-13684 (1985); Fryklund et al., Function and Structure of Parietal Cells After H^+, K^+-ATPase Blockade, Am. J. Physiol., 254 (3 PT 1); G399-407 (1988)]. A proton pump inhibitor may be selected for use in the compositions, conjugates, methods and uses disclosed herein based on one or more of the following properties: (i) a bioavailability greater than about 40%, 41%, 42%, 43%, 44%, 45%, 50%, 50% to 55%, 60%, 65%, 70%, 75%, 66%, 77%, 78%, 79%, 80%, 80% to 85%, 90%, 95%, 100%, 65-100%, or 80-95%; (ii) a plasma elimination half life greater than about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, or 3 hours, and (iii) it does not bind to cysteine 892 of the alpha subunit of the proton pump. In aspects of the invention, omeprazole, its salts, polymorphs, and any of its analogs and derivatives thereof are not encompassed within the term "proton pump inhibitor".

In aspects of the invention, a PPI includes compounds comprising a 2-[(2-pyridinyl) methylsulphinyl]-1H-benzimidazole skeleton or a related skeleton, which may optionally be substituted in various forms. A proton pump inhibitor may, if desired, be in the form of free base, free acid, salt, ester, solvates (in particular hydrates), anhydrate, amide, enantiomer, isomer, tautomer, prodrug, polymorph, derivative, or the like, provided that the free base, salt, ester, hydrate, amide, enantiomer, isomer, tautomer, prodrug, or any other pharmacologically suitable derivative is therapeutically active.

The following PPIs may be mentioned in the context of the present invention: 2-[2-(N-isobutyl-N-methylamino)benzyl-sulphinyl]benzimidazole (INN: leminoprazole) (DE-A-3531487); 2-(4-methoxy-6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-ylsulphinyl)-1H-benzimidazole (INN: nepaprazole) (EP-A-0 434 999); 2-(4-methoxy-3-methyl-pyridin-2-ylmethylsulphinyl)5-pyrrol-1-y-1H-benzimidazole (IY-81149), 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methylsulphinyl]-1-H-inidazo[4,5-b]pyridine(tenatoprazole), 2-[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl)methylsulphinyl]-1H-benzimidazole (INN: lansoprazole) (EP-A 0 174 726); and 2-{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]-methylsulphinyl}-1H-benzimidazole (INN: rabeprazole) (EP-A 0 184 322, EP-A 0 254 588, EP-A-0 261 478, EP-A-0 268 956); and in particular 5-difluoromethoxy-2-[(3,4-dimethoxy-2-pyridinyl)methylsulphinyl]-1H-benzimidazole (INN: pantoprazole) (EP-A-0 124 495, EP-A-0 166 287); and (−)-5-difluoromethoxy-2-[(3,4-dimethoxy-2-pyridinyl)methylsulphinyl]-1H-benzimidazole [(−)pantoprazole].

Other proton pump inhibitors include but are not limited to: soraprazan (Altana); ilaprazole (U.S. Pat. No. 5,703,097) (Il-Yang); AZD-0865 (AstraZeneca); dontoprazole; habeprazole; perprazole; ransoprazole; pariprazole; YH-1885 (PCT Publication WO 96/05177) (SB-641257) (2-pyrimidinamine, 4-(3,4-dihydro-1-

methyl-2(1H)-isoquinolinyl)-N-(4-fluorophenyl)-5,6-dimethyl-, monohydrochloride) (YuHan); phenylalkyl-amino derivatives of condensed carbapenem cpds (WO-A-9523149); BY-112 (Altana); SPI-447 (Imidazo(1,2-a)thieno(3,2-c)pyridin-3-amine,5-methyl-2-(2-methyl-3-thienyl) (Shinnippon); 3-hydroxymethyl-2-methyl-9-phenyl-7H-8,9-dihydro-pyrano(2,3-c)-imidazo(1,2-a)pyridine (PCT Publication WO 95/27714) (AstraZeneca); Pharmaprojects No. 4950 (3-hydroxymethyl-2-methyl-9-phenyl-7H-8,9-dihydro-pyrano(2,3-c)-imidazo(1,2-a)pyridine) (AstraZeneca, ceased) WO 95/27714; Pharmaprojects No. 4891 (EP 700899) (Aventis); Pharmaprojects No. 4697 (PCT Publication WO 95/32959) (AstraZeneca); H-335/25 (AstraZeneca); T-330 (Saitama 335) (Pharmacological Research Lab); Pharmaprojects No. 3177 (Roche); BY-574 (Altana); Pharmaprojects No. 2870 (Pfizer); AU-1421 (EP 264883) (Merck); AU-2064 (Merck); AY-28200 (Wyeth); Pharmaprojects No. 2126 (Aventis); WY-26769 (Wyeth); pumaprazole (PCT Publication WO 96/05199) (Altana); YH-1238 (YuHan); Pharmaprojects No. 5648 (PCT Publication WO 97/32854) (Dainippon); BY-686 (Altana); YM-020 (Yamanouchi); GYKI-34655 (Ivax); FPL-65372 (Aventis); Pharmaprojects No. 3264 (EP 509974) (AstraZeneca); nepaprazole (Toa Eiyo); HN-11203 (Nycomed Pharma); OPC-22575; pumilacidin A (BMS); saviprazole (EP 234485) (Aventis); SKandF-95601 (GSK, discontinued); Pharmaprojects No. 2522 (EP 204215) (Pfizer); S-3337 (Aventis); RS-13232A (Roche); AU-1363 (Merck); SKand F-96067 (EP 259174) (Altana); SUN 8176 (Daiichi Phama); Ro-18-5362 (Roche); ufiprazole (EP 74341) (AstraZeneca); Bay-p-1455 (Bayer); BY308; perprazole; [4-(2,2,2-trifluoroethoxy)-3-methyl-2-pyridyl]-methyl]sulfenamide; (Z)-5-methyl-2-[2-(1-naphthyl)ethenyl]-4-piperidinopyridine HCl; 2-(4-cyclohexyloxy-5-methyl pyridin-2-yl)-3-(1-naphthyl)-1-propanol; methyl 2-cyano-3-(ethylthio)-3-(methylthio)-2propenoate; 2-((4-methoxy-2-pyridyl)methylsulphinyl)-5-(1,1,2,2-tetrafluoroethoxy)-1H-benzimidazole sodium; 2-[[[4-(2,2,3,3,4,4,4-heptafluoro butoxy)-2-pyridyl]methyl)sulfinyl]-1H-thieno[3,4-d]imidazole; 2-[[[4-(2,2,2-trifluoro ethoxy)-3-methyl-2-pyridyl]methyl] sulfinyl]-1H-benzimidazole; 2-[[[4-(2,2,2-trifluoro ethoxy)-3-methyl-2-pyridyl]methyl] sulfinyl]-1H-benzimidazole; 2-methyl-8-(phenyl methoxy)-imidazo(1,2-A)-pyridine-3-acetonitrile; (2-((2-dimethylaminobenzyl)sulfinyl)-benzimidazole); 4-(N-allyl-N-methyl amino)-1-ethyl-8-((5-fluoro-6-methoxy-2-benzi midazolyl)sulfinylmethyl)-1-ethyl 1,2,3,4-tetrahydroquinolone; 2-[[(2-dimethylamino phenyl)methyl]sulfinyl]-4,7-dimethoxy-1H-benzimidazole; 2-[(2-(2-pyridyl)phenyl) sulfinyl)-1H-benzimidazole; (2-[(2-amino-4-methylbenzyl)sulfinyl]-5-methoxy benzo[d]imidazole; (4(2-methylpyrrol-3-yl)-2-guanid isothiazole); 4-(4-(3-(imidazole) propoxy)phenyl)-2phenylthiazole; (E)-2-(2-(4-(3-(dipropylamino)butoxy)phenyl)-ethenyl)benzoxazole; (E)-2-(2-(4-(3-(dipropylamino) propoxy)phenyl)ethenyl)-benzothiazole; Benzeneamine, 2-[[(5-methoxy-1H-benzimidazol-2-yl)sulfinyl]methyl)-4-methyl-; 2,3-dihydro-2-methoxycarbonylamino-1,2-benzisothiazol-3-one; 2-(2-ethyl aminophenylmethylsulfinyl)-5,6-dimethoxybenzimidazole; 2-methyl-8-(phenyl methoxy)imidazo[1,2-a)pyridine-3-acetonitrile; 3-amino-2-methyl-8-phenyl methoxy imidazo[1,2-a)-pyrazine HCl; 2-[[(3-chloro-4-morpholino-2-pyridyl)methyl]-sulfinyl)-5-methoxy-(1H)-benzinidazole; [3-butyryl-4-(2-methylphenylamino)-8-methoxy-quinoline); 2-indanyl 2-(2-pyridyl)-2-thiocarbamoylacetate HCl; 2,3-dihydro-2-(2-pyridinyl)-thiazolo (3,2-a)-benzimidazole; 3-cyanomethyl-2-methyl-8-(3-methyl-2-butenyloxy)-(1,2-a)imidazo pyridine; zinc L-carnosine; or, a free base,

free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, prodrug, or derivative of these compounds.

In certain aspects the proton pump inhibitor is selected from the group consisting of 2-[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl) methylsulphinyl]-1H-benzimidizole (lansoprazole), 2-{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]me thylsulphinyl}-1H-benzimidazole(rabeprazole), 5-difluoromethoxy-2-[(3,4-di-methoxy-2-pyridinyl)-methylsulphinyl]-1H-benzimidazole (pantoprazole) and the hydrates, solvates, salts, hydrates of the salts and solvates of the salts thereof.

In certain aspects the proton pump inhibitor is selected from the group consisting of 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methylsulphinyl]-1H-benzimidazole (omeprazole), 5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulphinyl]-1H-benzimidazole(esomeprazole), 2-[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl) methylsulphinyl]-1H-benzimidizole(lansoprazole), 2-{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulphinyl}-1H-benzimidazole(rabeprazole), 5-difluoromethoxy- 2-[(3,4-di-methoxy-2-pyridinyl)-methylsulphinyl]-1H-benzimidazole (pantoprazole) and the hydrates, solvates, salts, hydrates of the salts and solvates of the salts thereof.

Still other proton pump inhibitors contemplated by the present invention include those described in the following U.S. patents: U.S. Pat. Nos. 4,628,098; 4,689,333; 4,786,505; 4,853,230; 4,965,269; 5,021,433; 5,026,560; 5,045,321; 5,093,132; 5,430,042; 5,433,959; 5,576,025; 5,639,478; 5,703,110; 5,705,517; 5,708,017; 5,731,006; 5,824,339; 5,855,914; 5,879,708; 5,948,773; 6,017,560; 6,123,962; 6,187,340; 6,296,875; 6,319,904; 6,328,994; 4,255,431; 4,508,905; 4,636,499; 4,738,974; 5,690,960; 5,714,504; 5,753,265; 5,817,338; 6,093,734; 6,013,281; 6,136,344; 6,183,776; 6,328,994; 6,479,075; 6,559,167.

In aspects of the invention a proton pump inhibitor is in the form of a salt. A salt of a proton pump inhibitor may be prepared from formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, ftimaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, stearic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, embonic, methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, cyclohexylaminosulfonic, algenic, β-hydroxybutyric, galactaric and galacturonic acids.

In an embodiment, acid addition salts are prepared from the free base of a proton pump inhibitor using conventional methods involving reaction of the free base with a suitable acid. Suitable acids for preparing acid addition salts include without limitation organic acids, such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like, as well as inorganic acids, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like.

In another embodiment, an acid addition salt is converted to a free base by treatment with a suitable base. In a further embodiment, an acid addition salt is a halide salt, which is prepared using hydrochloric or

hydrobromic acids. In still another embodiment, the basic salt is an alkali metal salt, such as a sodium salt or copper salt.

Examples of salts of proton pump inhibitors include without limitation: a sodium salt form such as esomeprazole sodium, omeprazole sodium, rabeprazole sodium, pantoprazole sodium; or a magnesium salt form such as esomeprazole magnesium or omeprazole magnesium described in U.S. Pat. No. 5,900,424; a calcium salt form; or a potassium salt form such as the potassium salt of esomeprazole described in U.S. Pat. No. 6,511,996. Other salts of esomeprazole are described in U.S. Pat. Nos. 4,738,974 and 6,369,085. Salt forms of pantoprazole and lansoprazole are disclosed in U.S. Pat. Nos. 4,758,579 and 4,628,098, respectively.

In an embodiment, esters of proton pump inhibitors are utilized. An ester may be prepared by functionalization of hydroxyl and/or carboxyl groups which may be present within the molecular structure of the drug. In another embodiment, the esters are acyl-substituted derivatives of free alcohol groups, such as moieties derived from carboxylic acids of the formula -RCOOR' where R' is an alkyl group in particular a lower alkyl group. An ester can be converted to a free acid, if desired, by using conventional procedures such as hydrogenolysis or hydrolysis.

A proton pump inhibitor or its salts can be in a crystalline form. Crystals of a proton pump inhibitor may contain variable amounts of solvent. Therefore, the term "proton pump inhibitor" includes all solvates, in particular all hydrates, of the proton pump inhibitors and their salts. In particular aspects of the invention the proton pump inhibitor is a salt or hydrate including without limitation pantoprazole-sodium sesquihydrate [pantoprazole-sodium×1.5 H_2O], (−)-pantoprazole-sodium sesquihydrate, pantoprazole-magnesium dihydrate, omeprazole-magnesium, omeprazole-magnesium tetrahydrate, esomeprazole-magnesium and esomeprazole-magnesium tetrahydrate

In various aspects of the invention, the proton pump inhibitor is a substituted bicyclic aryl-imidazole, wherein the aryl group may be, for example, a pyridine, a phenyl, or a pyrimidine group which is attached to the 4- and 5-positions of the imidazole ring. Proton pump inhibitors comprising a substituted bicyclic aryl-imidazole include, but are not limited to, omeprazole, hydroxyomeprazole, esomeprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, perprazole, tenatoprazole, ransoprazole, pariprazole, leminoprazole, or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, prodrug, or derivative thereof. (See, e.g., *The Merck Index,* Merck & Co. Rahway, N.J. (2001)).

Substituted bicyclic aryl-imidazole compounds as well as their salts, hydrates, esters, amides, enantiomers, isomers, tautomers, polymorphs, prodrugs, and derivatives may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry. See, e.g., *March, Advanced Organic Chemistry: Reactions, Mechanisms and Structure,* 4th Ed. (New York: Wiley-Interscience, 1992); Leonard et al., *Advanced Practical Organic Chemistry,* (1992); Howarth et al.; *Core Organic Chemistry* (1998); and Weisermel et al., *Industrial Organic Chemistry* (2002).

A tautomer of a substituted bicyclic aryl-imidazole includes without limitation tautomers of omeprazole such as those disclosed in U.S. Pat. Nos. 6,262,085; 6,262,086; 6,268,385; 6,312,723; 6,316,020; 6,326,384;

6,369,087; and 6,444,689; and U.S. Publication No. 02/0156103. An example of an isomer of a substituted bicyclic aryl-imidazole is an isomer of omeprazole including but not limited to an isomer disclosed in: Oishi et al., Acta Cryst. (1989), C45, 1921-1923; U.S. Pat. No. 6,150,380; U.S. patent publication No. 02/0156284; and PCT Publication No. WO 02/085889.

An amide of a bicyclic aryl-imidazole compound may be prepared using techniques known to those skilled in the art or described in the pertinent literature. For example, an amide may be prepared from esters, using suitable amine reactants, or they may be prepared from an anhydride or an acid chloride by reaction with an amine group e.g., ammonia or a lower alkyl amine.

Suitable polymorphs include but are not limited to the polymorphs described in PCT Publication No. WO 92/08716, and U.S. Pat. Nos. 4,045,563; 4,182,766; 4,508,905; 4,628,098; 4,636,499; 4,689,333; 4,758,579; 4,783,974; 4,786,505; 4,808,596; 4,853,230; 5,026,560; 5,013,743; 5,035,899; 5,045,321; 5,045,552; 5,093,132; 5,093,342; 5,433,959; 5,464,632; 5,536,735; 5,576,025; 5,599,794; 5,629,305; 5,639,478; 5,690,960; 5,703,110; 5,705,517; 5,714,504; 5; 5,731,006; 5,879,708; 5,900,424; 5,948,773; 5,948,789; 5,997,903; 6,017,560; 6,123,962; 6,147,103; 6,150,380; 6,166,213; 6,191,148; 5,187,340; 6,268,385; 6,262,086; 6,262,085; 6,296,875; 6,316,020; 6,328,994; 6,326,384; 6,369,085; 6,369,087; 6,380,234; 6,384,059, 6,428,810; 6,444,689; 6,462,058; 6,903,122; 6,933,389; and 6,939,971.

In aspects of the invention, a proton pump inhibitor suitable for use in the invention is a benzimidazole compound, for example, a benzimidazole compound described in the following patent documents U.S. Pat. Nos. 4,045,563; 4,255,431; 4,359,465; 4,472,409; 4,508,905; 4,628,098; 4,738,975; 5,045,321; 4,786,505; 4,853,230; 5,045,552, and 5,312,824; EP-A-295603; EP-A-166287; EP-A-519365; EP5129; EP 174,726; EP 166,287; GB 2,163,747; and JP-A-59181277.

In particular aspects of the invention a proton pump inhibitor comprises or is selected from the group consisting of omeprazole, hydroxyomeprazole, esomeprazole, tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, or prodrug thereof.

In particular aspects of the invention a proton pump inhibitor comprises or is selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, or prodrug thereof.

A "histamine-2 receptor antagonist" or "H-2 antagonist" refers to a compound which blocks H-2 receptors, but does not have meaningful activity in blocking histamine-1 receptors. Selective H-2 antagonists include compounds which are disclosed in US Pat. Nos. 5,294,433, 5,364,616, and US Patent Application No. 20050042283, including without limitation cimetidine [Merck Index, 11th edition (1989), p. 354 (entry no. 2279) and Physicians' Desk Reference, 46th edition (1992), p. 2228]; etintidine (U.S. Pat. No. 4,112,234); ranitidine or its hydrochloride salt (AH-19065) [U.S. Pat. No. 4,128,658, Merck Index, 11th edition (1989), p. 1291 (entry no. 8126), and Physicians' Desk Reference, 46th edition (1992), p. 1063]; hydroxymethyl ranitidine; ranitidine

bismuth citrate (GR-122311, GR-122311X); AH-18801; N-cyano-N'-(2-(((5-((dimethylamino)methyl)-2-f-uranyl) methyl) thio)ethyl)-N"-methyl-guanidine; tiotidine (U.S. Pat. No. 4,165,378); ORF-17578 (U.S. Pat. No. 4,203,909); lupitidine (SKF-93479) (U.S. Pat. No. 4,234,588); donetidine (SKF-3574); famotidine (YM- 11170, MK-208) [Merck Index, 11th edition (1989), p. 617 (entry no. 3881), and Physicians' Desk Reference, 46th edition (1992), p. 1524]; roxatidine or rozatidine acetate [U.S. Pat. No. 4,293,557, Merck Index, 11 th edition (1989), p. 1316 (entry no. 8252)]; pifatidine; lamtidine (U.S. Pat. No. 4,318,913); BL-6548; BMY-25271; zaltidine (U.S. Pat. No. 4,374,843); nizatidine [U.S. Pat. No. 4,375,547, Merck Index, 11th edition (1989), p. 1052 (entry no. 6582), and Physicians' Desk Reference, 46th edition (1992), p. 1246)]; mifentidine and its hydrochloride salt [(U.S. Pat. No. 4,386,099, Merck Index, 11th edition (1989), p. 973 (entry no. 6108)]; ICIA-5165 (U.S. Pat. No. 4,165,377); BMY-25368 (SKF-94482) (U.S. Pat. No. 4,390,701); SYF-94482; ICI-162846 (U.S. Pat. No. 4,451,463); ramixotidine (U.S. Pat. No. 4,474,790); BL-6341A (BMY-26539) (U.S. Pat. No. 4,394,508); Wy-45727 (U.S. Pat. No. 4,490,527); SR-58042 (U.S. Pat. No. 4,514,408); BMY-25405 (U.S. Pat. Nos. 4,528,377 and 4,600,779); loxtidine (U.S. Pat. No. 4,536,508); DA-4634 (U.S. Pat. Nos. 4,548,944 and 4,645,841); bisfentidine (U.S. Pat. No. 4,649,150); sufotidine (U.S. Pat. No. 4,670,448); ebrotidine (U.S. Pat. No. 4,728,755); HE-30-256 (U.S. Pat. No. 4,738,960); D-16637 (U.S. Pat. No. 4,738,983); FRG-8813 (U.S. Pat. Nos. 4,912,101 and 4,977,267); FRG-8701 (U.S. Pat. No. 4,837,316); impromidine (U.K. Patent Specification No. 1,531,237); L-643728 (European Patent Application No. 0,040,696); MK-208 (U.S. Pat. No. 4,283,408). and HB-408 (European Patent Application No. 0,186,275); burimamide; and metiamide.

"Condition(s) and/or disease(s)" refers to one or more pathological symptoms or syndromes for which either or both of an exendin agonist or a gastrin compound provide a beneficial or therapeutic effect. The condition and/or disease may require reduction of blood glucose levels, inhibition of gastric acid secretion, increased pancreatic insulin content, increased pancreatic β-cell mass, inhibition of apoptosis of β-cells, stimulation of proliferation or differentiation of β-cells, and reduction of body weight. Examples of conditions and/or diseases include but are not limited to dyslipidemia, hyperglycemia, severe hypoglycemic episodes, stroke, left ventricular hypertrophy, arrhythmia, bacteraemia, septicaemia, irritable bowel syndrome, functional dyspepsia, diabetes, catabolic changes after surgery, stress induced hyperglycemia, respiratory distress syndrome, gastric ulcers, myocardial infarction, impaired glucose tolerance, hypertension, chronic heart failure, fluid retentive states, metabolic syndrome and related diseases and disorders, obesity, diabetic complications as well as symptoms of other diseases in which tissue is damaged due to elevated glucose levels, including Alzheimer's Disease, Parkinson's Disease, and other age-related, tissue-degenerative diseases, as well as the artherogenic effects of elevated leptin, for example in patients with impaired glucose tolerance and obese non-diabetic patients.

The term, "diabetes" as used herein means any manifested symptoms of diabetes in any mammal including experimental animal models, and including human forms such as Type 1 and Type 2 diabetes, early stage diabetes, and a pre-diabetic condition characterized by mildly decreased insulin or mildly elevated blood glucose levels. Diabetes disease processes may be derived from multiple causative factors and are characterized

by elevated levels of plasma glucose or hyperglycemia in the fasting state or after administration of glucose during an oral glucose tolerance test. Increased and premature morbidity and mortality are associated with persistent or uncontrolled hyperglycemia. Abnormal glucose homeostasis may be associated both directly and indirectly with alterations of lipid, lipoprotein and apolipoprotein metabolism and other metabolic and hemodynamic diseases.

Conditions and/or diseases associated with diabetes, particularly Type 2 diabetes mellitus, include but are not limited to diabetic nephropathy, diabetic retinopathy and diabetic neuropathy, macular degeneration, coronary heart disease, myocardial infarction, diabetic cardiomyopathy, myocardial cell death, coronary artery diseases, peripheral arterial disease, stroke, limb ischemia, vascular restenosis, foot ulcerations, endothelial dysfunction and/or atherosclerosis. In particular, patients with Type 2 diabetes mellitus may be at an increased risk of macrovascular and microvascular complications, including coronary heart disease, stroke, peripheral vascular disease, hypertension, nephropathy, neuropathy, and retinopathy.

A "pre-diabetic condition" describes a subject demonstrating a symptom in terms of insulin or glucose level, and/or demonstrating a susceptibility to diabetes or a related condition due to family history, genetic predisposition, or obesity in the case of Type 2 diabetes, and includes a subject who has previously had diabetes or a related condition and is subject to risk of recurrence.

In aspects of the invention, a condition and/or disease may be selected from the group consisting of (a) Type 1 or Type 2 diabetes mellitus and related diseases, disorders or conditions (including but not limited to diabetic nephropathy, diabetic retinopathy and diabetic neuropathy); (b) insulin resistance and syndrome X, obesity and related diseases, disorders or conditions (including but not limited to Insulin Resistance, Type 2 Diabetes Mellitus, Reproductive Disorders, Cardiovascular Disease, Pulmonary Disease, Gallstones and Fasting-induced cholecystitis, Cancers and Cutaneous Disease), Cushing's Syndrome, Hypothyroidism, Insulinoma, Craniopharyngioma and Other Disorders Involving the Hypothalamus; (c) congestive heart failure, left ventricular hypertrophy, survival post myocardial infarction (MI), coronary artery diseases, atherosclerosis, angina pectoris, thrombosis, (d) hypertension including hypertension in the elderly, familial dyslipidemichypertension and isolated systolic hypertension (ISH); increased collagen formation, fibrosis, and remodeling following hypertension (antiproliferative effect of the combination); impaired vascular compliance, stroke; all these diseases or conditions associated with or without hypertension, (e) renal failure, in particular chronic renal failure, glomerulosclerosis, nephropathy; (f) hypothyroidism; (g) endothelial dysfunction with or without hypertension, (h) hyperlipidemia, hyperlipoproteinemia, hypertryglyceridemia, and hypercholesterolemia, (i) macular degeneration, cataract, glaucoma, o) skin and connective tissue disorders, and (k) restenosis after percutaneous transluminal angioplasty, and restenosis after coronary artery bypass surgery; and peripheral vascular disease.

In particular aspects of the invention the condition and/or disease is diabetes, conditions of impaired glucose tolerance (IGT), conditions of impaired fasting plasma glucose, metabolic acidosis, ketosis, arthritis, obesity and osteoporosis, inflammatory bowel disease, Colitis Ulcerosa, Morbus Crohn, and/or metabolic

syndrome or B-cell protection, preferably an exendin agonist and a gastrin compound are used as therapeutic active substances for the treatment and/or prophylaxis of non-insulin dependent diabetes mellitus and/or impaired glucose tolerance.

"Insulinotropic activity" refers to an ability of a substance to stimulate insulin secretion in response to elevated glucose levels to produce or increase glucose uptake by cells and decreased serum glucose or blood glucose levels. Methods known in the art can be employed to assay for insulinotropic activity. For example, *in vitro* and *in vivo* methods may be used that measure insulin and/or C-peptide levels. Compounds, compositions or conjugates described herein have insulinotropic activity if islet cells secrete insulin in the presence of the compounds, compositions, or conjugates above background levels or levels in the absence of the compounds, compositions, or conjugates. A compound may be administered to an animal and the insulin concentration can be monitored over time.

"Islet neogenesis" means formation of new beta cells by differentiation, which may or may not have the characteristics of stem cells, which have the ability to reproduce in an unlimited manner.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The invention is related to compositions, conjugates, and methods that utilize an exendin agonist and a gastrin compound to provide beneficial effects. In particular, the invention relates to compositions, conjugates, and methods for the prevention, intervention and/or treatment of a condition and/or disease disclosed herein comprising an exendin agonist and a gastrin compound. In aspects of the invention, the compositions, conjugates and methods of the invention provide enhanced beneficial effects, in particular sustained beneficial effects relative to an exendin agonist and/or a gastrin compound alone. The beneficial effects may be additive or synergistic effects, preferably synergistic effects.

In aspects of the invention, where the condition and/or disease is diabetes, beneficial effects, in particular sustained beneficial effects of a composition, combination treatment, or conjugate of the invention may manifest as one or more of the following:

a) An increase in pancreatic insulin levels relative to the levels measured in the absence of an exendin agonist and a gastrin compound or for each compound alone after administration to a subject with symptoms of diabetes. In particular, the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% increase in pancreatic insulin levels in a subject. In aspects of the invention, the combination of an exendin agonist and a gastrin compound stimulate pancreatic insulin levels that approximate 65 to 95%, 70-95%, 75 to 90%, 80-90% or 90-100% of normal levels.

b) An increase in pancreatic insulin content and release into plasma relative to the amount measured in the absence of an exendin agonist and a gastrin compound or for each compound alone. In particular, the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%,

2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% increase in pancreatic insulin content and release into plasma.

c) A reduction of an absence of symptoms of islet inflammation after administration to a subject with symptoms of diabetes.

d) A decrease in blood glucose levels relative to the levels measured in the absence of the compounds or for each compound alone in subjects with symptoms of diabetes. Preferably, the compounds induce at least about a 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% decrease in blood glucose levels. Most preferably, the compounds yield blood glucose levels about or close to the levels common in a normal subject.

e) An increase in C-peptide levels relative to the levels measured in the absence of the compounds or for each compound alone in subjects with symptoms of diabetes. Preferably, the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in C-peptide levels.

f) Maintenance of blood glucose levels at about normal for a prolonged period of time.

g) Maintenance of hemoglobin A1c or glycated hemoglobin at about normal levels for a prolonged period of time, in particular maintaining a % hemoglobin A1c at between 6-8%, more particularly at about 7%.

h) A reduction in destruction of beta-cells. Preferably the compounds induce at least about a 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction.

i) An increase in beta-cell function. Preferably the compounds induce at least about a 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% increase in beta-cell function.

j) An increase in β-cell mass, in particular an increase in β-cell mass in pancreatic ducts. Preferably the compounds induce at least about a 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% increase in β-cell mass.

k) A reduction, prevention, or slowing of the rate of disease progression in a subject with diabetes.

l) A reduction or prevention of the development of ketoacidosis in patients with symptoms of diabetes.

m) A reduction or decrease in insulin delivery or usage compared with the absence of the compounds or for each compound alone in diabetic subjects. Preferably, the compounds provide at least about a 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 93%,94%, 95%, 96%, 97%, 98%, 99%, 100%, 30-100%, 30-80%, or 35-75%, reduction in insulin delivery or usage.

n) A reduction or prevention of the development of severe hyperglycemia.

o) An increase in survival in a subject with symptoms of diabetes.

In embodiments of the invention, beneficial effects or sustained beneficial effects comprise or consist essentially of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or thirteen of a) through m). In particular embodiments, beneficial effects or sustained beneficial effects comprise or consist essentially of a), b), and c); a), b), c), and d); a), b), c), d), and e); a), b), c), d), e), and f); a), b), c), d), e), f), and g); a), b), c), d), e), f), g), and h); a), b), c), d), e), f), g), h), and i); a), b), c), d), e), f), g), h), i) and j); a), d), and e); a), d), e), and h); a), d), e), h), and i); a), d), e), h), i), and j); a), b), c), d), e), h), i), and j); a), b), c), d), e), h), i), j), and k); b), c), d), and e); b), c), d), e), h), i), and j); b), h), i) and j); a) through e); a) through f); a) through g); a) through h); a) through i); a) through j); a) through k); a) through l); and a) through m), a) through n) and a) through o).

One or more of these beneficial effects or sustained beneficial effects can be demonstrated in a diabetic subject or disease model, for example a non-obese (NOD) mouse with symptoms of diabetes, using standard methods known to the skilled artisan. For example, commercially available methods and kits may be used to assay pancreatic insulin levels, glucose levels, C-peptide levels and hemoglobin A1c.

A gastrin compound may be selected for particular embodiments in the present invention and to provide a specific beneficial effect(s) based on characteristics including its insulinotrophic activity, the ability to augment the activity of an exendin agonist (in particular to enhance the insulinotropic effects of an exendin agonist), and/or increase the physical or chemical stability of an exendin agonist. A gastrin compound can also be selected based on its ability to stimulate proliferation/differentiation of beta cells, and its *in vivo* half-life.

In an aspect of the invention, a gastrin compound used in the methods, compositions, and conjugates of the invention is gastrin 17 and analogs and derivatives thereof. In a particular aspect, the gastrin compound is synthetic human gastrin I having 17 amino acid residues with a Leu residue at amino acid position 15 [SEQ ID NO. 18].

In another aspect of the invention, a gastrin compound used in the methods, compositions and conjugates of the invention is gastrin 34 and analogs and derivatives thereof. In a particular aspect, the gastrin compound is a synthetic human gastrin 34 with methionine or leucine at position 32 [SEQ ID NO. 15 or 16].

In particular aspects of the invention, a gastrin compound comprises synthetic human gastrin 34 having 2-34 amino acid residues of SEQ ID NO. 15 or 16, and optionally an N-terminal cysteine and/or a carrier; synthetic human gastrin having 1-17 amino acid residues with a Leu residue at amino acid position 15 [SEQ ID NO. 18] and optionally an N-terminal cysteine residue; and a synthetic human gastrin having amino acid residues 2 to 17 or 5-17 of SEQ ID NO. 17 or 18, optionally with an N-terminal cysteine residue and/or a carrier (e.g. PEG or human serum albumin) linked via a spacer [e.g. Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala i.e. $(GA)_5$] [SEQ ID NO. 26], in particular, a synthetic human gastrin having amino acid residues 2 to 17 or 5-17 of SEQ ID NO. 17 or 18, optionally with a human serum albumin (HSA) polymer linked via a Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala [ie. $(GA)_5$] spacer, and optionally an N-terminal cysteine residue.

In particular aspects of the invention the gastrin compound is a leucine substituted gastrin 17 of SEQ ID NO. 18. Such a gastrin compound may also be characterized by the following properties: isoelectric point of

about 3.4; purity of at least about 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and/or a molecular mass of about 2080.2 ±2 Da.

An exendin agonist may be selected for particular applications in the present invention based on one or more of the following characteristics: ability to initiate a signal transduction pathway resulting in insulinotropic activity; insulinotropic activity; stimulation of beta cell proliferation/differentiation; resistance to DP IV cleavage; and, an *in vivo* half-life of at least about 15 minutes to 24 hours, preferably 2 to 10 hours or 2 to 8 hours in humans using conventional methods (see for example, the method described in US 2003/0144206).

In embodiments of the invention, an exendin agonist is exendin (e.g. exendin 3 and exendin 4) or an analog, derivative, or fragment thereof. In another embodiment, the exendin agonist is a long acting exendin-3 or exendin-4. In embodiments of the invention, an exendin agonist is an insulinotropic analogue of exendin-4(1-39), in particular Ser^2Asp^3-exendin-4(1-39) wherein the amino acid residues in position 2 and 3 have been replaced with serine and aspartic acid, respectively.

In certain aspects of the invention the exendin agonist is a stable exendin agonist in particular a stable exendin analogue or derivative, or a stable exendin-4 or exendin-3 analogue or derivative.

In embodiments of the invention, the exendin agonist is exenatide, in particular injectable exenatide, most preferably Byetta®. In other embodiments, the exendin is a long-acting release formulation of exenatide.

In particular embodiments, an exendin agonist has the empirical formula $C_{184}H_{282}N_{50}O_{60}S$ and a molecular weight of 4186.6 daltons, and the following amino acid sequence: H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2 [SEQ. ID NO. 14].

Pharmaceutical compositions of the invention can be selected that provide beneficial effects, in particular statistically significant beneficial effects or sustained beneficial effects, compared with an exendin agonist or a gastrin compound alone. Beneficial effects in respect to a diabetic condition may be evidenced by one or more of the beneficial effects described herein, in particular one, two, three, four, five, six, seven, eight, nine or ten of the beneficial effects described above in a) through o).

The invention provides a pharmaceutical composition with beneficial effects, in particular statistically significant beneficial effects or sustained beneficial effects comprising exendin-4 and gastrin-17(leu) [SEQ ID NO. 18].

The invention provides a pharmaceutical composition with beneficial effects, in particular statistically significant beneficial effects or sustained beneficial effects comprising exenatide and gastrin-17(leu) [SEQ ID NO. 18].

The invention also provides a pharmaceutical composition with beneficial effects, in particular statistically significant beneficial effects or sustained beneficial effects, comprising an exendin agonist selected from the group consisting of exendin-3, exendin-4 or analogues or derivatives thereof, in particular H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2, and a gastrin compound having an amino acid

sequence comprising, from the amino terminus, Z-Y_m-X_n-AA_1-AA_2-AA_3-AA_4-AA_5-AA_6, wherein AA_1 is Tyr or Phe, AA_2 is Gly, Ala, or Ser, AA_3 is Trp, Val, or Ile, AA_4 is Met or Leu, AA_5 is Asp or Glu, and AA_6 is Phe or Tyr; Z is a polymer and when the polymer is a protein Z is an amino acid sequence; Y_m is an optional spacer region comprising m amino acid residues of a small neutral amino acid including but not limited to serine and alanine, and X is any consecutive portion of residues 1-28 of SEQ ID NO: 15 or 16, or residues 1-11 of SEQ ID NO. 17 or 18, preferably AA_1-AA_2-AA_3-AA_4-AA_5-AA_6 is Tyr-Gly-Trp-Met-Asp-Phe or Tyr-Gly-Trp-Leu-Asp-Phe. In a particular embodiment, Z is a serum protein, in particular human serum albumin.

In certain aspects of the invention, pharmaceutically acceptable salts of an exendin agonist and/or pharmaceutically acceptable salts of a gastrin compound are utilized.

The invention in particular aspects provides a pharmaceutical composition which has been adapted for administration to a subject to provide sustained beneficial effects to treat a condition and/or disease, preferably diabetes. In an embodiment for the prevention and/or treatment of diabetes, the composition is in a form such that administration to a subject results in blood glucose levels that are about normal that persist for a prolonged period of time after cessation of treatment, increased pancreatic insulin content and release into plasma, increased pancreatic β-cell mass, stimulation of β-cell regeneration, and/or increased C-peptide production.

This invention provides a conjugate comprising an exendin agonist linked to or interacting with a gastrin compound wherein the interaction is for example, via an amino or a carboxyl group. The invention also relates to isolated covalent conjugates of the invention, and compositions comprising covalent conjugates of the invention. An exendin agonist may be conjugated to a species via an ester bond between an OH and a COOH of a gastrin compound. Conjugates of an exendin agonist and a gastrin compound may be conjugated with an intermediate spacer or linker. A suitable spacer or linker may be a mono- or disaccharide, an amino acid, a sulfate, a succinate, an acetate, or an oligomeric polymeric spacer or linker comprising one or more of such moieties.

The invention also provides methods of preparing conjugates that result in conjugates with improved pharmacokinetic properties, biological activity, and/or beneficial effects. The methods comprise incubating the exendin agonist with a gastrin compound under conditions that allow formation of a covalent linkage between the two compounds. The invention therefore contemplates a process for preparing a covalent conjugate comprising an exendin agonist covalently bonded or linked to a gastrin compound, the process comprising: incubating the exendin agonist with a gastrin compound under conditions and at a pH and for a time sufficient for formation of a covalent bond or linkage between the exendin agonist and gastrin compound; and isolating the covalent conjugate. The above process for preparing a conjugate comprising an exendin agonist and a gastrin compound may provide a conjugate with a substantial amount of an exendin agonist covalently linked to the exendin agonist.

N-terminal or C-terminal fusion proteins or chimeric proteins, comprising an exendin agonist conjugated with a gastrin compound, optionally with a spacer or linker, may also be prepared by fusing, through

recombinant techniques, the N-terminal or C-terminal sequence of an exendin agonist and the sequence of a gastrin compound.

The invention relates to a conjugate prepared by a process described herein. The invention also relates to pharmaceutical formulation or composition comprising conjugates of the invention and a pharmaceutically acceptable carrier, excipient, or vehicle. The invention further relates to a pharmaceutical formulation or composition of substantially pure covalent conjugates comprising an exendin agonist covalently linked to a gastrin compound which provides beneficial effects preferably sustained beneficial effects compared to the exendin agonist alone. In an embodiment, a pharmaceutical formulation is provided consisting essentially of covalent conjugates comprising an exendin agonist covalently linked without an intermediate spacer or linker to a gastrin compound. In another embodiment, a pharmaceutical formulation is provided consisting essentially of covalent conjugates comprising an exendin agonist covalently linked with an intermediate spacer or linker to a gastrin compound.

In aspects of the invention, a composition or conjugate is provided comprising an exendin agonist and a gastrin compound having greater sustained insulinotropic activity following treatment compared with the activity of an exendin agonist alone.

The invention provides methods for the prevention, treatment and/or intervention of a condition and/or disease in a subject comprising administering a gastrin compound and an exendin agonist or a pharmaceutical composition of the invention to provide a beneficial effect, in particular a sustained beneficial effect.

In methods of the invention providing beneficial effects, in particular statistically significant beneficial effects or sustained beneficial effects, an exendin agonist is an exendin-4 agonist, in particular, H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2, or a long acting analogue or derivative thereof.

In certain methods of the invention exendin-4 agonist and gastrin-17(leu) [SEQ ID NO. 18] are administered. In other aspects of the invention, exenatide and gastrin-17(leu) [SEQ ID NO. 18] are administered.

In certain further methods of the invention providing beneficial effects, in particular statistically significant beneficial effects or sustained beneficial effects, an exendin agonist is an exendin-4, in particular, H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2, and a gastrin compound comprises an amino acid sequence comprising, from the amino terminus, Z-Y_m-X_n-AA_1-AA_2-AA_3-AA_4-AA_5-AA_6, wherein AA_1 is Tyr or Phe, AA_2 is Gly, Ala, or Ser, AA_3 is Trp, Val, or Ile, AA_4 is Met or Leu, AA_5 is Asp or Glu, and AA_6 is Phe or Tyr; Z is a polymer and when the polymer is a protein Z is an amino acid sequence; Y_m is an optional spacer region comprising m amino acid residues of a small neutral amino acid including but not limited to serine and alanine, and X is any consecutive portion of residues 1-28 of SEQ ID NO: 15 or 16, or residues 1-17 of SEQ ID NO. 17 or 18, preferably AA_1-AA_2-AA_3-AA_4-AA_5-AA_6 is Tyr-Gly-Trp-Met-Asp-Phe or Tyr-Gly-Trp-Leu-Asp-Phe. In a particular embodiment, Z is a serum protein, in particular human serum albumin.

In an aspect, the invention provides a method for the prevention and/or intervention of a condition and/or disease discussed herein in a subject comprising administration of at least one exendin agonist and at least one gastrin compound. An exendin agonist and a gastrin compound may be directly administered to a subject or contacted with cells (e.g. stem cells or progenitor cells) and administered to a subject.

The invention also provides a combination treatment for preventing and/or treating a condition and/or disease discussed herein in a subject comprising administering to the subject a therapeutically effective amount of at least one exendin agonist and a gastrin compound which provides beneficial effects following treatment. In an aspect the invention provides a combination treatment or intervention which provides sustained beneficial effects following treatment. The invention also relates to a method of treatment comprising administering a therapeutically effective amount of at least one exendin agonist in combination with the administration of at least one gastrin compound which upon administration to a subject with symptoms of diabetes produces beneficial effects, preferably sustained beneficial effects, manifested as about normal blood glucose levels, reduced insulin use, increased β-cell mass, and/or increased pancreatic insulin. In an aspect of the invention therapeutically effective amounts of an exendin agonist and a gastrin compound are combined prior to administration to a subject. In an embodiment, therapeutically effective amounts of an exendin agonist and a gastrin compound are mixed at a physiologically acceptable pH.

In an aspect, the invention provides a method for stimulating beta cell proliferation in a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination an exendin agonist and a gastrin compound. In another aspect, the invention provides a method for increasing the number and/or size of beta cells in a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention or administering in combination an exendin agonist and a gastrin compound.

In a further aspect, the invention provides a method for preventing or treating Type 1 or Type 2 diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination an exendin agonist and a gastrin compound.

In a still further aspect, the invention provides a method for ameliorating progression of disease or obtaining a less severe stage of disease in a person suffering from Type 1 diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination an exendin agonist and a gastrin compound.

In a still further aspect, the invention provides a method for ameliorating progression of disease or obtaining a less severe stage of disease in a person suffering from Type 2 diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination an exendin agonist and a gastrin compound.

The invention relates to a method of delaying the progression of impaired glucose tolerance or non-insulin requiring Type 2 diabetes to insulin requiring Type 2 diabetes comprising administering a therapeutically

effective amount of a composition or conjugate of the invention, or administering in combination an exendin agonist and a gastrin compound.

The invention also relates to a method of increasing the insulin synthesis capability of a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination an exendin agonist and a gastrin compound.

The invention further relates to inducing islet neogenesis in a subject comprising contacting islet precursor cells with an exendin agonist and a gastrin compound, composition, or conjugate of the invention in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

The invention contemplates a method of expanding a functional beta cell mass of pancreatic islet transplants in a diabetic patient, the method comprising administering to the patient a therapeutically effective amount of an exendin agonist and a gastrin compound, or a composition or conjugate of the invention.

In an aspect, the invention provides methods for treating diabetes mellitus in a patient in need thereof by administering a composition comprising a gastrin compound and an exendin agonist in an amount sufficient to effect differentiation of the patient's pancreatic islet precursor cells to mature insulin-secreting cells and/or to stimulate insulin synthesis in existing islet cells. The composition can be administered systemically or expressed *in situ* by host cells containing one or more nucleic acid construct in an expression vector wherein the nucleic acid construct comprises a coding sequence for a gastrin compound or a coding sequence for an exendin agonist or for both compounds, together with transcriptional and translational regulatory regions functional in pancreatic islet precursor cells.

In an aspect, the invention provides a method for treating diabetes in a subject receiving insulin, and one or more glucose lowering agent comprising administering a therapeutically effective amount of an exendin agonist and a gastrin compound. In an embodiment of the invention, the invention provides a method for treating diabetes in a subject receiving insulin, and one or more glucose lowering agent comprising administering a therapeutically effective amount of an exendin agonist and a gastrin compound. In particular embodiments, the glucose lowering agents are a biguanide compound, a thiazolidinedione, or an α-glucosidase inhibitor, preferably metformin and a thiazolidinedione. In particular aspects, the exendin agonist is exenatide and the gastrin compound is a gastrin-17(leu) [SEQ ID NO. 18].

The invention provides methods for treating cells, preferably cells in culture using an exendin agonist and gastrin compound of the invention, or compositions, or conjugates of the invention. The invention also provides cell based treatment methods using an exendin agonist and a gastrin compound of the invention, or compositions, or conjugates of the invention. See PCT/CA03/33595 for a description of general culture and cell based treatment methods.

In an aspect, the invention relates to a method for expanding and differentiating stem cells or progenitor cells into insulin secreting cells comprising contacting the stem cells or progenitor cells with an exendin agonist and a gastrin compound or a composition or conjugate of the invention in sufficient amounts to expand and differentiate stem cells or progenitor cells. The amount of expansion and differentiation may be significantly

different compared with that achieved in the absence of the compounds, composition or conjugate, in particular the amount may be significantly greater compared with an amount achieved with an exendin agonist or a gastrin compound alone. In an embodiment, the stem cells or progenitor cells are contacted with the compounds, composition, or conjugate in culture. In another embodiment, the stem cells or progenitor cells are contacted with the compounds, composition, or conjugate in a subject. The compounds, composition or conjugate may be administered to a subject before, during, or after implantation of stem cells in the subject to expand and differentiate the stem cells in the subject. The stem cells may be obtained from pancreatic islets, umbilical cords, embryos, or stem cell lines. The method may additionally comprise administering an immunosuppressive agent.

The invention also relates to a method for enhancing proliferation of insulin secreting cells in culture comprising contacting the cells with an exendin agonist and a gastrin compound, composition or conjugate of the invention in sufficient amounts to enhance proliferation of the cells. The amount of proliferation may be significantly different compared with that achieved in the absence of the compounds, composition or conjugate. In an embodiment, the amount of proliferation is significantly greater compared with an exendin agonist or a gastrin compound alone

The invention further relates to a method for sustaining islet cells or precursor cells in culture comprising culturing the cells in the presence of an exendin agonist and a gastrin compound, composition, or conjugate of the invention in an amount sufficient to sustain the cells in culture. The cells may be sustained in culture for a significantly longer period of time compared with cells cultured in the absence of the compounds, composition or conjugate, or in the presence of an exendin agonist or a gastrin compound alone. Culturing cells in the presence of an exendin agonist and a gastrin compound or a composition or conjugate of the invention will be particularly useful in preparing and maintaining cells intended for transplantation.

In an aspect, the invention provides a method of treating a condition and/or disease comprising administering an exendin agonist and a gastrin compound, a composition or conjugate of the invention with a plurality of cells to a subject in need thereof to thereby produce a beneficial effect, preferably a sustained beneficial effect.

A method for treating a subject with a condition and/or disease described herein comprises contacting *ex vivo* a plurality of cells with an exendin agonist and a gastrin compound, or a composition or conjugate of the invention of the invention, optionally culturing the cells, and administering the cells to the subject in need thereof.

In embodiments of the aforementioned cell based therapeutic methods the cells are pancreatic ductal cells and the amount of compounds, composition, or conjugate used in the method is generally effective to increase the amount of insulin secreting cells in the subject. The cells may be autologous (i.e. from the same subject), or may be from another individual of the same species, or from a different species.

The invention also contemplates a method for treating diabetes in a subject comprising transplanting a pancreatic islet preparation into the subject and administering a therapeutically effective amount of an exendin agonist and a gastrin compound, or a composition or conjugate of the invention.

In the cell based methods of the invention the number of cells administered to an individual afflicted with a condition and/or disease will vary according to the severity of the condition and/or disease, the mode of administration, and/or the site of administration. Generally a therapeutically effective amount of cells is a safe and effective amount, and in particular an amount necessary to provide one or more beneficial effect, in particular a sustained beneficial effect, or a synergistic effect.

Cells can be administered to subjects using a variety of means apparent to those of skill in the art. Suitable methods include injection of the cells into a target site in a subject. Cells may be inserted into a delivery device to facilitate injection or implantation into the subjects. Examples of delivery devices include tubes, e.g., catheters, for injecting cells and fluids into the body of a subject. Cells can be prepared for delivery in a variety of different forms. For example, the cells may be suspended in a solution or gel, or mixed with a pharmaceutically acceptable carrier, excipient, or diluent in which the cells remain viable. Pharmaceutically acceptable carriers, excipients, and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is generally sterile, and will often be isotonic. A solution of cells is preferably selected that is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms through the use of, for example, parabens, chlorobutanol, phenol, scorbic acid, thimerosal, and the like.

Modes of administration of cells include without limitation systemic intracardiac, intracoronary, intravenous, intradermal, or intra-arterial injection and injection directly into the tissue or organ at the intended site of activity, or in proximity to the site of activity. A cell preparation can be administered by any convenient route, for example by infusion or bolus injection and can be administered together with other biologically active agents. Administration in some aspects is preferably systemic.

Methods of the invention may further comprise measuring or monitoring one or more of the following markers: blood glucose, serum glucose, blood glycosylated haemoglobin, pancreatic beta cell mass, serum insulin, pancreatic insulin levels, morphometrically determined beta cell mass, amount of insulin secreting cells, and glucose responsiveness of insulin secreting cells.

The invention also contemplates the use of a composition comprising a combination of at least one exendin agonist and at least one gastrin compound for the preparation of a medicament providing beneficial effects, preferably sustained beneficial effects in treating a condition and/or disease. In an aspect, the invention relates to the use of a therapeutically effective amount of at least one exendin agonist and at least one gastrin compound for preparation of a medicament for providing beneficial effects, preferably sustained beneficial effects, in treating a condition and/or disease. In an embodiment the invention provides the use of an exendin agonist and a gastrin compound for the preparation of a medicament for increasing (preferably sustained increase) the number and/or size of beta cells or pancreatic β-cell mass in a subject after treatment. In another embodiment the invention provides the use of an exendin agonist and a gastrin compound for the preparation of a medicament for stimulation (preferably sustained stimulation) of beta cell proliferation after treatment. In a still

further embodiment the invention provides the use of a exendin agonist and a gastrin compound for the preparation of a medicament for treatment of Type 1 or Type 2 diabetes.

The invention additionally provides uses of a pharmaceutical composition and a conjugate of the invention in the preparation of medicaments for beneficial effects, preferably sustained beneficial effects, in the treatment of conditions and/or diseases.

Therapeutic efficacy and toxicity of compounds, compositions and conjugates of the invention may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals such as by calculating a statistical parameter such as the ED_{50} (the dose that is therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The therapeutic index is the dose ratio of therapeutic to toxic effects and it can be expressed as the ED_{50}/LD_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred.

The compounds, compositions, medicaments, and conjugates of the present invention can be administered by any means that produce contact of the active agent(s) with the agent's sites of action in the body of a subject or patient. The active ingredients can be administered simultaneously or sequentially, and in any order at different points in time, to provide the desired beneficial effects. The compounds, conjugates and compositions can be formulated for sustained release, for delivery locally or systemically. It lies within the capability of a skilled physician or veterinarian to select a form and route of administration that optimizes the effects of the compositions, conjugates, and treatments of the present invention.

In accordance with aspects of the present invention, an exendin agonist and a gastrin compound may be administered in any effective order or time interval. However, the factors are preferably "concurrently administered," meaning that independent of the order in which the factors are administered, the factors are administered within a time interval such that the effect of the factors is at least greater than additive. The factors may also be administered together. In accordance with the present invention, each factor may be independently administered any effective number of times, including more than once, as may be indicated by a physician or veterinarian.

The compounds, compositions and conjugates may be administered in oral dosage forms such as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. They may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular forms, all utilizing dosage forms well known to those of ordinary skill in the pharmaceutical arts. The compounds, compositions and conjugates of the invention may be administered by intranasal route via topical use of suitable intranasal vehicles, or via a transdermal route, for example using conventional transdermal skin patches. A dosage protocol for administration using a transdermal delivery system may be continuous rather than intermittent throughout the dosage regimen.

A particular route of administration is parenteral administration, preferably peripheral parenteral administration. Parenteral administration is generally understood to refer to the injection of a dosage form into the body by a sterile syringe or some other mechanical device such as an infusion pump. For the purpose of the

present invention parenteral routes include intravenous, intramuscular, subcutaneous, and intraperitoneal routes of administration. For parenteral administration, the compounds, compositions or conjugates described herein may be combined with distilled water at an appropriate pH.

The present invention includes combination treatments providing additive or synergistic activity, or that deliver an additive or synergistically effective amount, or an amount to provide a therapeutically effective amount of an exendin agonist and a gastrin compound, or a conjugate or composition of the invention. Therefore, pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in a synergistically effective amount or a therapeutically effective amount.

The dosage regimen of the invention will vary depending upon known factors such as the pharmacodynamic characteristics of the agents and their mode and route of administration; the species, age, sex, health, medical condition, and weight of the patient, the nature and extent of the symptoms, the kind of concurrent treatment, the frequency of treatment, the route of administration, the renal and hepatic function of the patient, and the desired effect. The effective amount of a drug required to prevent, counter, or arrest progression of a condition and/or disease can be readily determined by an ordinarily skilled physician or veterinarian.

A composition, medicament, or treatment of the invention may comprise a unit dosage of at least one exendin agonist and a unit dosage of at least one gastrin compound. A "unit dosage" refers to a unitary i.e. a single dose which is capable of being administered to a patient, and which may be readily handled and packed, remaining as a physically and chemically stable unit dose comprising either the active agents as such or a mixture with one or more solid or liquid pharmaceutical excipients, carriers, or vehicles.

In an aspect, a pharmaceutical composition or method is provided comprising a therapeutically effective suboptimal dosage of an exendin agonist and a gastrin compound that are more effective at decreasing or reducing glucose levels, increasing β-cell mass, stimulating β-cell regeneration, increasing C-peptide, and/or increasing pancreatic insulin production for a sustained period following treatment compared with a dosage of either a gastrin compound or exendin agonist alone.

In another aspect, an improved pharmaceutical composition or method is provided comprising therapeutically effective suboptimal amounts of an exendin agonist and a gastrin compound in a form for chronic or acute therapy of a condition and/or disease, in particular diabetes.

In an embodiment, a composition or method comprises an exendin agonist and a gastrin compound in doses that are equal to or at least 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold lower than the doses of each compound required to provide beneficial effects, preferably sustained beneficial effects, to treat a condition and/or disease.

In an aspect the invention provides a pharmaceutical composition or method comprising between 0.5 to 6000, 0.5 to 4000, 0.5 to 3000, 0.5 to 2000, 0.5 to 1000, 0.5 to 500, 0.5 to 200, 0.5 to 100, 0.5 to 50, 0.5 to 25, 0.5 to 15, 1 to 15, 1 to 10, or 5 to 10 micrograms exendin agonist per single unit and 0.5 to 8000, 0.5 to 7000, 0.5 to 6000, 100-3000, 100-6000, 1000-6000, 1000 to 5000, 1000 to 4000, 2000-6000, and 3000-6000 micrograms gastrin compound per single unit.

In another aspect the invention provides a pharmaceutical composition or method comprising between 0.01 to 20, 0.01 to 10, 0.01 to 5, 0.01 to 3, 0.01 to 2, 0.01 to 1, 0.01 to 0.5, 0.01 to 0.3, 0.01 to 0.2, 0.02 to 1, 0.02 to 0.5, 0.02 to 0.3, 0.02 to 0.2, 0.03 to 1, 0.03 to 0.5, 0.03 to 0.3, 0.03 to 0.2, 0.04 to 1, 0.04 to 0.5, 0.04 to 0.3, 0.04 to 0.2, 0.05 to 1, 0.05 to 0.5, 0.05 to 0.3, 0.05 to 0.2, 0.06 to 1, 0.06 to 0.5, 0.06 to 0.3, 0.06 to 0.2, 0.07 to 1, 0.07 to 0.5, 0.07 to 0.3, 0.07 to 0.2, or 0.07 to 0.15 micrograms/kg/day exendin agonist and 1 to 100, 1 to 90, 1 to 80, 1 to 75, 1 to 70, 1 to 50, 1 to 40, 1 to 30, 5 to 100, 5 to 80, 5 to 70, 5 to 50, 5 to 40, 5 to 30, 10 to 100, 10 to 90, 10 to 80, 10 to 75, 10 to 70, 10 to 50, 10 to 40, 10 to 30, 15 to 90, 15 to 80, 15 to 75, 15 to 70, 15 to 50, 15 to 40, 15 to 30, or 15 to 25 micrograms/kg/day gastrin compound.

In another aspect the invention provides a pharmaceutical composition or method comprising between 0.01 to 10, 0.01 to 1, 0.01 to 0.5, 0.01 to 0.2, 0.01 to 0.1, 0.01 to 0.15, or 0.07 to 0.15 micrograms/kg/day exendin agonist (e.g. exenatide) and 1 to 100, 10 to 90, 10 to 80, 10 to 75, 15 to 90, 15 to 80, 15 to 75, or 14 to 71 micrograms/kg/day gastrin compound [e.g. gastrin- 17(leu)].

In aspects of compositions and methods of the invention between about 0.1 to 10 mg, 0.2 to 5 mg, 0.4 to 5 mg, 0.5 to 5mg, 0.6 to 5 mg, 0.7 to 5mg, 0.5 to 3 mg, 0.7 to 2mg, or 0.8 to 2 mg of a long-acting release formulation of an exendin agonist, in particular a long-acting release formulation of exenatide, can be employed. In particular a long-acting release formulation of exenatide (e.g. Byetta®) in a dose of about 0.5 mg to 5 mg, more particularly 0.5 mg to 3 mg, most particularly 0.8 mg to 2.0 mg may be administered at least once weekly, preferably once-weekly subcutaneously.

The compounds or a composition or formulation of the invention may be administered to a subject continuously for 2 weeks to 12 months, 2 weeks to 6 months, 2-16 weeks, 2 weeks to 12 weeks, and/or 2-8 weeks, or periodically.

An exendin agonist and a gastrin compound may be in a ratio selected to augment the activity of one or both compounds to produce beneficial effects, in particular a sustained beneficial effect, and/or to produce an additive or synergistic effect. In embodiments, the ratio of an exendin agonist to a gastrin compound may be from 1:1 to 1:200, 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, 1:1 to 1:5, and 1:1, in particular 1:100. In other embodiments, the ratio of a gastrin compound to an exendin agonist may be from 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, and 1:1 to 1:5. In a particular embodiment the ratio of an exendin agonist to a gastrin compound is from 1:50 to 1:150, more particularly 1:100.

An exendin agonist may be used in combination with a gastrin compound at therapeutically effective weight ratios of between about 1:1 to 1:200, 1:1 to 1:150, 1:1 to 1:100, or 1:1 to 1:50. In another embodiment, a gastrin compound may be used in combination with an exendin agonist at therapeutically effective weight ratios of between about 1:1 to 1:200, 1:1 to 1:150, 1:1 to 1:100, or 1:1 to 1:50.

Methods, conjugates and compositions of the invention may employ the doses of exendin agonist and gastrin compound disclosed herein.

The compositions of the present invention or fractions thereof typically comprise suitable pharmaceutical diluents, excipients, vehicles, or carriers selected based on the intended form of administration,

and consistent with conventional pharmaceutical practices. The carriers, vehicles etc. may be adapted to provide additive, synergistically effective or therapeutically effective amounts of the active compounds. Suitable pharmaceutical diluents, excipients, vehicles, and carriers are described in the standard text, Remington: The Science and Practice of Pharmacy, 21st Edition. University of the Sciences in Philadelphia (Editor). By way of example, for oral administration in the form of a capsule or tablet, the active components can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, methyl cellulose, magnesium stearate, glucose, calcium, sulfate, dicalcium phosphate, mannitol, sorbital, and the like. For oral administration in a liquid form, the drug components may be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Suitable binders (e.g. gelatin, starch, corn sweeteners, natural sugars including glucose; natural and synthetic gums, and waxes), lubricants (e.g. sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, and sodium chloride), disintegrating agents (e.g. starch, methyl cellulose, agar, bentonite, and xanthan gum), flavoring agents, and coloring agents may also be combined in the compositions or components thereof.

In an aspect of the invention a pharmaceutical composition has a pH from about 7 to 10.

Formulations for parenteral administration of a composition of the invention may include aqueous solutions, syrups, aqueous or oil suspensions and emulsions with edible oil such as cottonseed oil, coconut oil or peanut oil. Dispersing or suspending agents that can be used for aqueous suspensions include synthetic or natural gums, such as tragacanth, alginate, acacia, dextran, sodium carboxymethylcellulose, gelatin, methylcellulose, and polyvinylpyrrolidone.

Compositions for parenteral administration may include sterile aqueous or non-aqueous solvents, such as water, isotonic saline, isotonic glucose solution, buffer solution, or other solvents conveniently used for parenteral administration of therapeutically active agents. A composition intended for parenteral administration may also include conventional additives such as stabilizers, buffers, or preservatives, e.g. antioxidants such as methylhydroxybenzoate or similar additives.

In an embodiment, a solid form pharmaceutical composition is provided (e.g. tablets, capsules, powdered, or pulverized form) comprising a crystalline or amorphous exendin agonist and a crystalline or amorphous gastrin compound.

In another embodiment, the invention relates to a liquid drug formulation comprising pharmaceutically acceptable salts of an exendin agonist and a gastrin compound, and to lyophilized drug formulations that can be reconstituted to provide suspensions that are stable and suitable for parenteral administration.

In a particular embodiment, the invention relates to an aqueous composition comprising pharmaceutically acceptable salts of an exendin agonist and a gastrin compound, and a solvent system which effects solubilization. The invention also provides a drug comprising an aqueous formulation of pharmaceutically acceptable salts of an exendin agonist and a gastrin compound with at least one solubilizer.

The compounds, conjugates and compositions of the invention may be sterilized by, for example, filtration through a bacteria retaining filter, addition of sterilizing agents to the composition, irradiation of the

composition, or heating the composition. Alternatively, the compounds, conjugates, and compositions of the present invention may be provided as sterile solid preparations e.g. lyophilized powder, which are readily dissolved in sterile solvent immediately prior to use.

In addition to the formulations described herein, the compositions can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the fractions may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil), or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions of the invention and components thereof may comprise soluble polymers as targetable drug carriers.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labelled for treatment of an indicated condition. For administration of a composition of the invention, such labelling would include amount, frequency, and method of administration.

Since the present invention relates to a method of treatment comprising a combination of active agents which may be administered separately or as conjugates, the invention also provides a kit comprising an exendin agonist and a gastrin compound, a pharmaceutical composition or conjugate in kit form. The invention also relates to a pharmaceutical kit comprising one bottle with an exendin agonist and another bottle with a gastrin bottle in one box. A kit may comprise a package which houses a container which contains a conjugate or composition of the invention and also houses instructions for administering the conjugate or composition to a subject.

In aspects of the invention, a pharmaceutical pack or kit is provided comprising one or more containers filled with one or more of the ingredients of a pharmaceutical composition of the invention to provide a beneficial effect, in particular a sustained beneficial effect. Associated with such container(s) can be various written materials such as instructions for use, or a notice in the form prescribed by a governmental agency regulating the labeling, manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use, or sale for human administration.

In an aspect, the invention relates to a "kit-of-parts", for example, the components to be combined according to the present invention can be dosed independently or by use of different fixed combinations with distinguished amounts of the components, i.e. simultaneously or at different time points. The parts of the kit can then be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit. Time intervals can be selected such that the effect on the condition and/or disease in the combined use of the parts is larger than the effect that would be obtained by use of any one of the components.

In an aspect the invention provides a kit of parts comprising: (a) an amount of an exendin agonist or a pharmaceutically acceptable salt thereof in a first unit dosage; and (b) an amount of a gastrin compound or a

pharmaceutically acceptable salt thereof in a second unit dosage, in the form of one or two separate units of the components (a) and (b).

The invention further relates to a commercial package comprising at least one exendin agonist and at least one gastrin compound, together with instructions for simultaneous, separate or sequential use. In an aspect a commercial package comprising as active ingredients at least one exendin agonist and at least one gastrin compound is provided in the form of two or more separate units of the components, together with instructions for its simultaneous, separate or sequential use, or any combination thereof, in the delay of progression or treatment of a condition and/or disease disclosed herein.

The present invention also includes compositions, kits and methods of using the compositions and kits of the invention in combination with one or more additional therapeutic agents including without limitation immunosuppressive agents, antidiabetic agents including without limitation insulin sensitivity enhancers, glucose lowering agents, insulin secretagogues, insulin, antiobesity agents, appetite regulating drugs, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with a condition and/or disease, in particular diabetes and obesity, anti-nausea, anti-headache medications, and general medications that treat or prevent side effects.

In aspects of the invention, an antidiabetic compound is an insulin signalling pathway modulator, such as inhibitors of protein tyrosine phosphatases (PTPases), non-small molecule mimetic compounds and inhibitors of glutamine-fructose-6-phosphate amidotransferase (GFAT), compounds influencing a dysregulated hepatic glucose production, like inhibitors of glucose-6-phosphatase, inhibitors of fructose-1,6-bisphosphatase, inhibitors of glycogen phosphorylase, glucagon receptor antagonists and inhibitors of phosphoenolpyruvate carboxykinase, pyruvate dehydrogenase kinase inhibitors, insulin secretagogues including sulfonylureas, meglinitides, and amylin modulators, insulin sensitivity enhancers, insulin secretion enhancers, alpha-glucosidase inhibitors, inhibitors of gastric emptying, insulin, and alpha 2-adrenergic antagonists. In particular aspects, the additional therapeutic agent is nateglinide, repaglinide, metformin, rosiglitazone, pioglitazone, troglitazone, glisoxepid, glyburide, glibenclamide, acetohexamide, chloropropamide, glibornuride, tolbutamide, tolazamide, glipizide, carbutamide, gliquidone, glyhexamide, phenbutamide, tolcyclamide, glimepiride and gliclazide, a peroxisome proliferator-activated receptor α compound, or a pharmaceutically acceptable salt of such a compound.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

Example 1

Effects of Gastrin ± Exendin-4 in Acutely-Diabetic NOD Mice

A study was conducted in an animal model of human autoimmune Type 1 diabetes, a non-obese diabetic (NOD) mouse, to assess the efficacy of gastro-intestinal peptides to induce neogenesis of insulin-producing pancreatic islet β-cells and correct hyperglycemia. Exendin (Byetta®) was obtained from Amylin/Eli

Lilly Pharmaceuticals, and synthetic human Gastrin I [17 leu 15] was obtained from Transition Therapeutics Inc. NOD mice were treated daily with the combination of Byetta® (exenatide) at 3 μg/kg and Gastrin at 10 and 30 μg/kg.

Female (diabetes-prone) NOD mice were monitored for diabetes onset from age 10 weeks by urine testing for glucosuria; diabetes was confirmed by detecting the fasting blood glucose (FBG) to be > 8 mM.

Treatments were started within one week of diabetes onset (FBG = 10-16 mM), and were administered I.P. in 0.2 ml, twice daily (8 a.m. and 6 p.m.) for 3 weeks. The mice were monitored daily for urine glucose and weekly for body weight and FBG levels. FBG levels were measured at 8 a.m., 12 hours after food had been withdrawn and 14 hours after the last treatment injection. All treatments were stopped at 3 weeks and the mice continued to be monitored daily for glucosuria and weekly for body weight and FBG levels.

PBS-vehicle-treated NOD mice develop severe hyperglycemia (FBG >25-30 mM) with ketosis and weight loss, and had to be sacrificed at 4-8 weeks after entry into the study (entry is within 1 week of diabetes onset, FBG = 10-16 mM). Therefore, the study was terminated at 8 weeks; i.e., after 3 weeks ON treatments + 5 weeks of follow-up OFF treatment. At 8 weeks, mice from all groups were sacrificed for the following studies. Blood was collected for FBG, total glycosylated hemoglobin, and plasma C-peptide levels. Pancreata was collected for assay of insulin content, and histological examination. Mice that develop severe hyperglycemia (FBG >25-30 mM with weight loss) before 8 weeks of study were sacrificed, and blood and pancreata were collected and examined as above.

The results are illustrated in Figures 1 to 7. In particular, Figures 1 to 4 show that a combination therapy of exenatide and gastrin corrects hyperglycemia in NOD mice. Figure 5 shows that a combination therapy with exenatide and gastrin restores pancreatic insulin content in NOD mice. Figure 6 shows that a combination therapy with exenatide and gastrin prevents loss of insulin secretion (plasma C-peptide) after diabetes onset in NOD mice. Figure 7 shows that blood glucose levels in NOD mice treated with exenatide ± gastrin are inversely correlated with pancreatic insulin content.

The results show that a combined exendin and gastrin treatment to diabetic NOD mice normalized hyperglycemia to effectively treat the diabetes, and it had a prolonged effect on fasting blood glucose levels indicating a stimulation of beta cell neogenesis and insulin production.

Example 2

Effect of G1/GLP-1 in the NOD Mouse Model

The non-obese diabetic (NOD) mouse model is the most widely used animal model of Type 1 diabetes. In this model, β-cells are selectively destroyed by cell-mediated autoimmunity. This is the standard animal model used to evaluate whether a treatment will be effective in the presence of active β-cell autoimmunity. The studies in the NOD mouse model presented below tested the effects of G1 in combination with native GLP-1 as well as a longer acting analogue, exenatide.

G1 is a synthetic human peptide consisting of 17 amino acids and is the same length as the native gastrin. G1 contains a single amino acid change at position 15, where leucine replaces methionine to enhance molecular stability. The amino acid sequence of G1 is as follows:

Single Letter Format: Pyr*-GPWLEEEEEAYGWLDF

Three Letter Format

1 Pyr* gly pro trp leu glu glu glu glu glu

11 ala tyr gly trp leu asp phe stop

Where *Pyr = Pyr-OH = pGlu = Glp = pyroglutamic acid (cyclic Glu)

A. Effects of G1 and GLP-1 in the NOD Mouse Model:

This study examined the effects of G1 in combination with GLP-1 on acutely diabetic NOD mice. The treatment was initiated with either vehicle or with 300 μg/kg/day of GLP-1 in combination with 3 μg/kg/day of G1 by ip. After 18 days of daily treatments, in animals treated with the combination of 300 μg/kg/day of GLP-1 and 3 μg/kg/day of G1, fasting blood glucose (FBG) was 6.1 ± 0.7 mM, whereas the FBG was 24.4 ± 1.5 mM in the vehicle treated group (Figure 8). In comparison, the animals treated with GLP-1 alone had FBG levels of 12.5 ± 2.2 mM. These data indicate that G1 and GLP-1 combination treatment was more effective than GLP-1 alone in controlling glucose levels in NOD mice. FBG levels were monitored weekly for an additional six weeks post treatment. One week following the completion of treatment, FBG levels were normal (below 6mM) in mice injected with the combination therapy and remained as such through the end of the study with FBG of 4.3 ± 0.2 mM at six weeks post treatment. Comparatively, animals treated with GLP-1 reached high blood glucose levels and suffered from diabetic complications (Figure 8).

Non-diabetic mice have approximately 10 μg of insulin per pancreas, whereas acutely diabetic animals with elevated glucose levels have 0.5 to 1.0 μg of pancreatic insulin, showing that NOD mice require less than 10% of their pancreatic insulin to regulate glucose levels. Five weeks following the onset of diabetes the untreated animals had minimal levels of pancreatic insulin, and at this stage the animals had glucose levels of 30-32 mM and suffered from diabetic complications. The GLP-1 treated group had pancreatic insulin levels of 1.0 to 1.5 μg, which is higher than untreated animals suggesting that GLP-1 stimulates some islet cell regeneration in the NOD mouse model.

Strikingly, the G1 and GLP-1 treated animals had over 8 μg of insulin per pancreas which is significantly higher than GLP-1 alone but represents close to 80% of normal non-diabetic pancreatic insulin levels (Figure 9). These studies show that the G1 and GLP-1 combination treatment is robust in stimulating islet cell regeneration that is capable of reversing disease for long periods of time post treatment. G1 and GLP-1 were able to restore pancreatic insulin content from the low levels measured after diabetes onset and before treatment to a level similar to that measured in normoglycemic mice. Correction of hyperglycemia in NOD mice was significantly correlated with the increase in pancreatic insulin content ($r = 0.90$).

Histological examination as shown in Figure 10 revealed large, intensely insulin-stained islets adjacent to pancreatic ducts and surrounded but not invaded by mononuclear leukocytes in GLP-1 and G1 treated mice.

Insulin stained cells (in dark brown), are few in acutely diabetic NOD mice before treatment. The number of these islet cells decrease further in the untreated group over time. The β-cell mass decreased from 0.41 mg to 0.01 mg during the course of the experiment, whereas the β-cell mass increased to 1.05 mg in the group of animals treated with G1 and GLP-1. When examined 6 weeks after completion of treatment, mice still showed elevated β-cell mass. The combination treatment therefore provides a significantly increased and sustained β-cell mass in NOD mice.

B. Effect of G1 and the GLP-1 Analogue Exenatide (Byetta®) in the NOD Mouse Model

The studies in the NOD mouse model presented below tested the effects of G1 in combination with a exenatide, a long acting analogue of GLP-1.

NOD mice were treated daily with the combination of Byetta® (exenatide) at 3 μg/kg and Gastrin at 10 and 30 μg/kg. After 3 weeks of treatment there was a significant improvement in FBG levels and pancreatic insulin content in animals exposed to the combination treatment vs. animals exposed to exenatide alone (Figures 2 and 5). The data clearly show the potentiating effects on hyperglycemia reversal by adding G1 to Exenatide. The blood glucose level in NOD mice treated with the combination therapy showed significant correlation with the pancreatic insulin content ($r = -0.88$).

These studies show that the G1 and GLP-1 combination treatment in the NOD mouse model is robust in stimulating islet cell regeneration and is capable of reversing disease for long periods of time post treatment. The G1 and GLP-1 were able to restore pancreatic insulin content from the low levels measured after diabetes onset and before treatment to a level similar to that measured in normoglycemic mice. The combination therapy with gastrin and native GLP-1 or exenatide is more effective than therapies with the peptides given as single agents.

Example 3

Effect of G1 and GLP-1 on Human Pancreatic Cells

The strong scientific evidence of replication and neogenesis of new β-cells in adult rat and mice models is well documented. To determine whether G1/GLP-1-islet neogenesis therapy can induce differentiation of human β-cells that are functional, a unique animal model system was used. In this model immunoincompetent mice are transplanted with human islet cells and exposed to systemic treatment with G1 and GLP-1 molecules. The studies presented below tested human islet cell preparations that are routinely used for human transplants by transplanting them into nondiabetic NOD-scid mice through implantation under the kidney capsule. Neogenesis of human islet cells as well as β- cell mass expansion and β-cell function under physiological stimulus were assessed.

This study investigated whether the β-cell mass in human pancreatic islet cell preparations with low endocrine cell purity could be expanded by treating the cells with G1 and GLP-1. Islets isolated from pancreata of adult human organ donors (5×10^6 cells) composed of 7% insulin+ β-cells, 28% duct cells and 24% acinar cells were implanted under the renal capsule in NOD-scid mice. Diabetes was induced with streptozotocin (blood glucose 20-25 mM). The mice were injected sc twice daily for 5 weeks with GLP-1 at 100 μg/kg/day and G1 at 300 μg/kg/day. After 5 weeks of treatment, blood glucose rose to 28.6 mM in vehicle-treated mice, while it

remained at ~ 20 mM in GLP-1 or G1-treated mice. In animals treated with the combination therapy glucose levels decreased to 14.7 mM whereas β-cells in the human pancreatic cell implants increased almost 5-fold. Insulin content in the human pancreatic cell implants in the mice increased from 20 ± 2 ng to 30 ± 5 ng after 5 weeks in vehicle-treated mice, 58 ± 19 ng in GLP-1-treated mice, 85 ± 28 ng in G1-treated mice and 424 ± 180 ng in GLP-1 and G1-treated mice.

Similarly, the plasma levels of human C-peptide (as a measure of human circulating insulin levels) in the mice increased from 29 ± 7 pmol/mL in vehicle-treated mice to 98 ± 28 pmol/mL in GLP-1-treated mice, to 171 ± 27 pmol/mL in G1-treated mice and to 258 ± 62 pmol/mL in GLP-1 plus G1-treated mice. Correction of hyperglycemia by the peptides correlated significantly with plasma human C-peptide levels ($r = -0.821$, $P < 0.001$). These results show that combination therapy with G1 and GLP-1 expands the β-cell mass of human pancreatic cell preparations implanted in diabetic NOD-scid mice 5-fold and this is sufficient to ameliorate hyperglycemia in the mice.

The data in Examples 2 and 3 demonstrate that systemic administration of G1 in combination with GLP-1 and its analogue exenatide effectively stimulate β-cell regeneration through β-cell neogenesis, resulting in increased pancreatic β-cell mass, pancreatic insulin content and insulin release into plasma in both Type 1 and Type 2 animal models for diabetes.

Therapeutic treatment with the combination therapy in acutely diabetic NOD mice prevented development of severe hyperglycemia by increasing pancreatic insulin levels and β-cell mass. The regeneration was able to out balance the islet destruction in this animal model. A relatively short course of G1 and GLP-1 treatment of diabetic NOD mice normalizes hyperglycemia and has a prolonged effect on FBG levels for periods of at least 6 weeks post treatment. In addition the data show that the G1 and GLP-1 combination is capable of stimulating pancreatic insulin levels that approximate 80-90% of normal levels, whereas GLP-1 alone only induces regeneration slightly. Furthermore, histological analysis of the pancreas shows that the islet cells appear normal and with large numbers of insulin producing cells, despite being surrounded by inflammatory cells. Morphometric analysis of the pancreas shows that G1 and GLP-1 treatment increases β-cell mass in the pancreas, and shows signs of inducing neogenesis by increasing β-cell mass in pancreatic ducts.

In summary, preclinical pharmacology studies show that G1 in combination with GLP-1 and its longer acting analogues are potent inducers of islet cell regeneration with long-lasting blood glucose-modulating properties.

Example 4

A phase 1 multiple ascending dose study to evaluate the safety, tolerability and pharmacokinetic profile of G1 in combination with Byetta® in healthy volunteers

Study Design: This is a single site multiple ascending dose study of G1 + Byetta® in healthy volunteers (n=24). Subjects will be assigned to one of 3 dose cohorts and will receive study drug for 7 days. Dose levels will be escalated between cohorts, such that the first cohort will receive the lowest dose level, the second cohort an

intermediate dose level, and the third cohort the highest proposed dose level. Dose escalation will be dependent on a review of safety data from the previously dosed cohort.

G1 will be escalated in each of the 3 cohorts while the Byetta® dose will remain fixed throughout the study. Three doses of G1 will be evaluated in three different subject cohorts so that each subject will receive only one dose level of study drug for 7 days. Each cohort will include 8 subjects.

Byetta® will be administered subcutaneously twice daily for 7 days as per the Byetta® Product Monograph at 10 µg per dose. G1 will be administered subcutaneously once daily for 7 days. The starting G1 dose will be 1 mg followed by planned doses of 2 mg and 4 mg.

Subjects will be screened up to 14 days prior to admission to the Phase 1 unit. Subjects will be admitted to the Phase 1 unit on Day 0, the day before dosing. Subjects will be dosed starting on Day 1 and will receive G1 once a day and Byetta® twice a day for 7 days.

Study Treatments: Subjects will be assigned to 1 of 3 dosing cohorts:

Cohort 1 = 1 mg G1 once daily + 10 µg Byetta® bid

Cohort 2 = 2 mg G1 once daily + 10 µg Byetta® bid

Cohort 3 = 4 mg G1 once daily + 10 µg Byetta® bid

Safety Endpoints: Incidence of AEs and SAEs

- Laboratory safety tests (hematology, biochemistry, urinalysis)
- Vital signs
- ECG
- Physical examination
- Concomitant medications

PK Endpoints: Plasma concentrations of G1 and Byetta® (AUC, C_{max}, t_{max}, $t_{1/2}$, K_{el})

Example 5

A phase 2 study to evaluate the safety, tolerability, pharmacokinetics and effects of G1 in combination with Byetta® in Type 2 diabetics

Study Design: This is a randomized double-blind, controlled study with three treatment arms. To be eligible for the study, Type 2 diabetics must have been on a stable Byetta® regimen for at least 3-6 months. Patients will continue to take Byetta® for the duration of the study. Upon successful completion of the screening phase, patients will be randomized to receive either:

1. Vehicle control in combination with their existing Byetta® regimen
2. Low Dose G1 (1 mg dose) in combination with their existing Byetta® regimen
3. High Dose G1 (2 mg dose) in combination with their existing Byetta® regimen

Twenty patients will be randomized to each treatment group, for a total of 60 patients. The Treatment Phase is 3 months in duration. Dose levels of G1 will be established in the study described in Example 4. Patients who complete the treatment phase will be followed for 3 months.

Byetta® will be administered subcutaneously twice daily for the duration of the trial (Baseline, Treatment and Follow-up) days as per the Byetta® Product Monograph. G1 will be administered subcutaneously once daily during the 3-month Treatment Phase only.

Study Treatments: Patients stable on Byetta® will be randomized to 1 of 3 treatment groups:

Group 1 = Vehicle control

Group 2 = Low dose G1 (1 mg dose)

Group 3 = High dose G1 (2 mg dose)

Safety Endpoints: Incidence of AEs and SAEs

- Laboratory safety tests (hematology, biochemistry, urinalysis)
- Vital signs
- ECG
- Physical examination
- Concomitant medications
- G1 and Byetta® antibodies
- Glycemic control (episodes of hypoglycemia)

Effects Endpoints: Beta cell function

- Hemoglobin A_{1c}
- Blood glucose levels
- C-peptide
- Other biomarkers including immune reactivity markers

PK Endpoints: Plasma concentrations of G1 and Byetta® (AUC, C_{max}, t_{max}, $t_{1/2}$, K_{el})

Example 6

A phase 2 study to evaluate the safety, tolerability, pharmacokinetics and effects of G1 in combination with Byetta® in Type 1 diabetics

Study Design: This is a randomized double-blind, controlled study with three treatment arms. To be eligible for the study, Type 1 diabetics must have been on a stable insulin regimen for at least 1 year. Upon successful completion of the Screening Phase, patients will be randomized to receive either:

1. Vehicle control
2. Low Dose G1 (1 mg dose) in combination with Byetta® regimen
3. High Dose G1 (2 mg dose) in combination with Byetta® regimen

Twenty patients will be randomized to each treatment group, for a total of 60 patients. The treatment phase is 3 months in duration. Dose levels of G1 will be established in the study described in Example 4. Patients who complete the treatment phase will be followed for 3 months.

Byetta® will be administered subcutaneously twice daily for the duration of the trial (Baseline, Treatment and Follow-up) days as per the Byetta® Product Monograph. G1 will be administered subcutaneously once daily during the 3-month Treatment Phase only.

Study Treatments: Patients stable on insulin will be randomized to 1 of 3 treatment groups:

Group 1 = Vehicle control

Group 2 = Low dose G1 (1 mg dose)

Group 3 = High dose G1 (2 mg dose)

Safety Endpoints: Incidence of AEs and SAEs

- Laboratory safety tests (hematology, biochemistry, urinalysis)
- Vital signs
- ECG
- Physical examination
- Concomitant medications
- G1 and Byetta® antibodies
- Glycemic control (episodes of hypoglycemia)

Effects Endpoints: Insulin use

- Beta cell function
- Hemoglobin A_{1c}
- Blood glucose levels
- C-peptide
- Other biomarkers including immune reactivity markers

PK Endpoints: Plasma concentrations of G1 and Byetta® (AUC, C_{max}, t_{max}, $t_{1/2}$, K_{el})

The present invention is not to be limited in scope by the specific embodiments described herein, since such embodiments are intended as but single illustrations of one aspect of the invention and any functionally equivalent embodiments are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising or consisting essentially of therapeutically effective amounts of an exendin agonist and a gastrin compound that provides beneficial effects relative to an exendin agonist alone, and a pharmaceutically acceptable carrier, excipient, or vehicle.

2. A pharmaceutical composition as claimed in claim 1 in a form that provides normal or reduced blood glucose levels in a subject that persist for a prolonged period of time after administration.

3. A pharmaceutical composition as claimed in claim 1 wherein the therapeutically effective amounts are suboptimal relative to the amount of each compound administered alone for treatment of diabetes.

4. A pharmaceutical composition as claimed in any preceding claim wherein the ratio of exendin agonist to gastrin compound is selected to augment the activity of the exendin agonist.

5. A pharmaceutical composition as claimed in claim 1 wherein the ratio of an exendin agonist to a gastrin compound is from about 1:1 to 1:200, 1:1 to 1:150, 1:1 to 1:110, or 1:1 to 1:100.

6. A pharmaceutical composition as claimed in any preceding claim wherein the exendin agonist and the gastrin compound are present in doses that are at least about 1.1 to 1.4, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold lower than the doses of each compound alone required to treat a condition and/or disease.

7. A pharmaceutical composition as claimed in claim 1 wherein the therapeutically effective amount is a synergistically effective amount.

8. A pharmaceutical composition as claimed in claim 1 comprising between 0.01 to 20, 0.01 to 10, 0.01 to 5, 0.01 to 3, 0.01 to 2, 0.01 to 1, 0.01 to 0.5, 0.01 to 0.3, 0.01 to 0.2, 0.02 to 1, 0.02 to 0.5, 0.02 to 0.3, 0.02 to 0.2, 0.03 to 1, 0.03 to 0.5, 0.03 to 0.3, 0.03 to 0.2, 0.04 to 1, 0.04 to 0.5, 0.04 to 0.3, 0.04 to 0.2, 0.05 to 1, 0.05 to 0.5, 0.05 to 0.3, 0.05 to 0.2, 0.06 to 1, 0.06 to 0.5, 0.06 to 0.3, 0.06 to 0.2, 0.07 to 1, 0.07 to 0.5, 0.07 to 0.3, 0.07 to 0.2, or 0.07 to 0.15 micrograms/kg/day exendin agonist and 1 to 100, 1 to 90, 1 to 80, 1 to 75, 1 to 70, 1 to 50, 1 to 40, 1 to 30, 5 to 100, 5 to 80, 5 to 70, 5 to 50, 5 to 40, 5 to 30, 10 to 100, 10 to 90, 10 to 80, 10 to 75, 10 to 70, 10 to 50, 10 to 40, 10 to 30, 15 to 90, 15 to 80, 15 to 75, 15 to 70, 15 to 50, 15 to 40, 15 to 30, or 15 to 25 micrograms/kg/day gastrin compound.

9. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effects are one or more of the following: stimulation of β-cell regeneration, increase in pancreatic β cell mass, prolonged increase in C-peptide production, increase in pancreatic insulin production, increase in pancreatic insulin content and insulin release into plasma, and/or about normal blood glucose levels, compared with exendin alone.

10. A pharmaceutical composition as claimed in claim 9 wherein the beneficial effects are sustained beneficial effects that persist for a prolonged period of time after termination of treatment.

11. A pharmaceutical composition as claimed in claim 10 wherein the beneficial effects are sustained for at least about 4, 6, or 10 weeks following treatment.

12. A pharmaceutical composition as claimed in claim 11 wherein the sustained beneficial effects may manifest as increased C-peptide production, increased pancreatic insulin production, increased β-cell mass and/or about normal or low blood glucose levels for a prolonged period following treatment.

13. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effect is at least about a 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in pancreatic insulin levels.

14. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effect is at least about a 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% decrease in blood glucose levels.

15. A pharmaceutical composition as claimed in any preceding claim wherein the exendin agonist comprises a sequence of any one of SEQ. ID NOs. 1 to 14.

16. A pharmaceutical composition as claimed in any preceding claim wherein the gastrin compound is gastrin 71, gastrin 52, gastrin 34 (big gastrin), gastrin 17 (little gastrin), gastrin 14, gastrin 8, gastrin 6, pentagastrin, and tetragastrin, or an analogue or derivative thereof.

17. A pharmaceutical composition as claimed in any preceding claim where the exendin agonist is exenatide.

18. A pharmaceutical composition as claimed in any preceding claim wherein the exendin agonist comprises a sequence of SEQ. ID NO. 14.

19. A pharmaceutical composition as claimed in any preceding claim wherein the gastrin compound is gastrin- 17(leu).

20. A conjugate comprising an exendin agonist linked to a gastrin compound.

21. A method for treating or preventing diabetes in a subject comprising administering to the subject a therapeutically effective amount of an exendin agonist and a gastrin compound, or a composition or conjugate of any preceding claim, to produce a sustained beneficial effect.

22. A method as claimed in claim 21 wherein the sustained beneficial effect is a decrease in blood glucose levels for a period of at least 2, 4, 6, 8, or 10 weeks, 2 to 4 weeks, 2 to 6 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment.

23. A method of treatment comprising administering to a subject a therapeutically effective amount of at least one exendin agonist in combination with administration of at least one gastrin compound which upon administration to a subject with symptoms of diabetes provides sustained beneficial effects.

24. A method as claimed in claim 23 wherein administration with of at least one exendin agonist in combination with administration of at least one gastrin compound provides sustained beneficial effects of at least one symptom of diabetes.

25. A method as claimed in claim 23 or 24 wherein therapeutically effective amounts of the exendin agonist and the gastrin compound are combined prior to administration to the subject.

26. A method as claimed in claim 23 or 24 wherein therapeutically effective amounts of the exendin agonist and the gastrin compound are administered to the subject sequentially.

27. A method as claimed in any preceding claim wherein the therapeutically effective amounts of an exendin agonist and a gastrin compound are synergistically effective amounts.

28. A method of treating diabetes comprising administering an exendin agonist and a gastrin compound, or a composition or conjugate of any preceding claim with a plurality of cells to a subject in need thereof to thereby produce beneficial effects, preferably sustained beneficial effects.

29. A method of any preceding claim wherein the diabetes is Type 1 diabetes.

30. A method any preceding claim wherein the diabetes is Type 2 diabetes.

31. A method for inducing islet neogenesis in a subject comprising contacting islet precursor cells with an exendin agonist and a gastrin compound, or a composition, or conjugate of any preceding claim in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

32. A method for expanding and differentiating stem cells into insulin secreting cells comprising contacting the stem cells with an effective amount of an exendin agonist and a gastrin compound or a composition or conjugate of any preceding claim.

33. Use of a composition comprising or consisting essentially of a combination of at least one exendin agonist and at least one gastrin compound for the preparation of a medicament for the treatment of diabetes.

34. Use of an exendin agonist for the manufacture of a medicament for the treatment of diabetes to be used in combination with a gastrin compound.

35. A kit form of a composition or conjugate as claimed in any preceding claim.

Figure 1

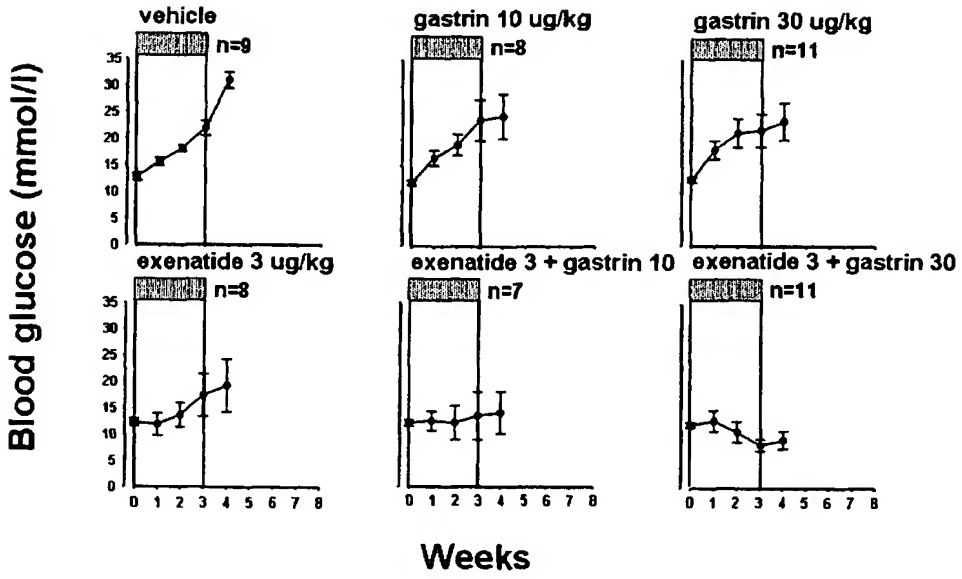

vehicle
n=9
gastrin 10 ug/kg
n=8
gastrin 30 ug/kg
n=11
exenatide 3 ug/kg
n=8
exenatide 3 + gastrin 10
n=7
exenatide 3 + gastrin 30
n=11
Blood glucose (mmol/l)
0 5 10 15 20 25 30 35
0 1 2 3 4 5 6 7 8
Weeks

Figure 2

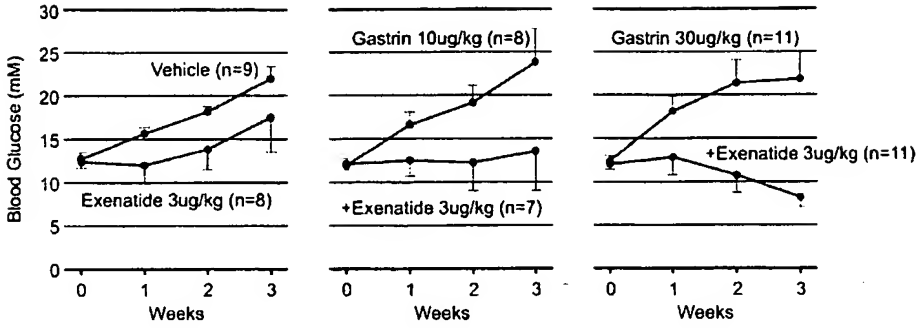

Blood Glucose (mM)
30
25
20
15
10
5
0
Vehicle (n=9)
Exenatide 3ug/kg (n=8)
Gastrin 10ug/kg (n=8)
+Exenatide 3ug/kg (n=7)
Gastrin 30ug/kg (n=11)
+Exenatide 3ug/kg (n=11)
0 1 2 3
Weeks
0 1 2 3
Weeks
0 1 2 3
Weeks

Figure 3

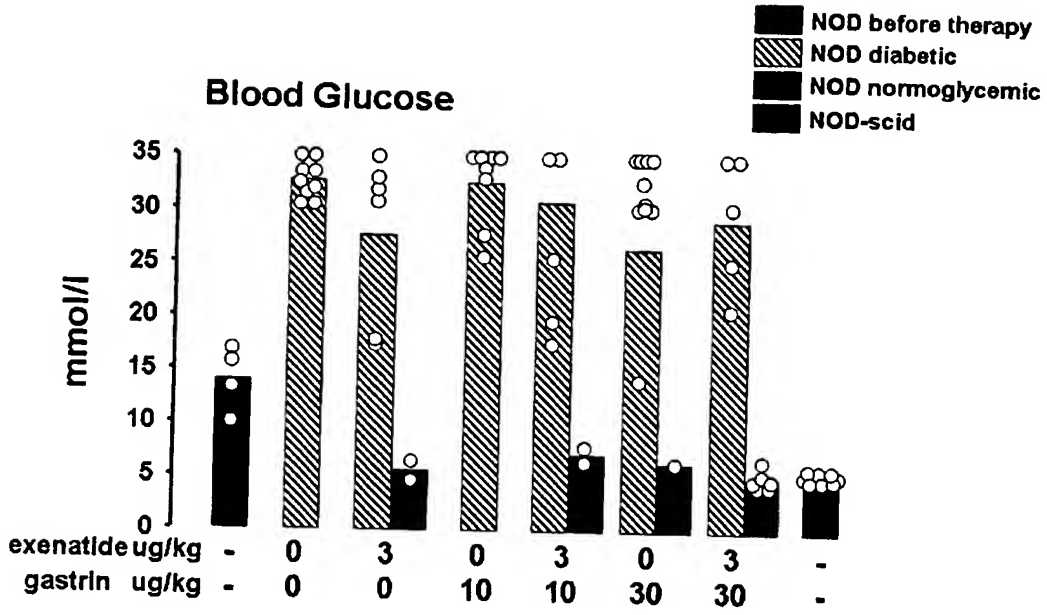

NOD before therapy
NOD diabetic
NOD normoglycemic
NOD-scid
Blood Glucose
mmol/l
35
30
25
20
15
10
5
0
exenatide ug/kg - 0 3 0 3 0 3 -
gastrin ug/kg - 0 0 10 10 30 30 -

Figure 4

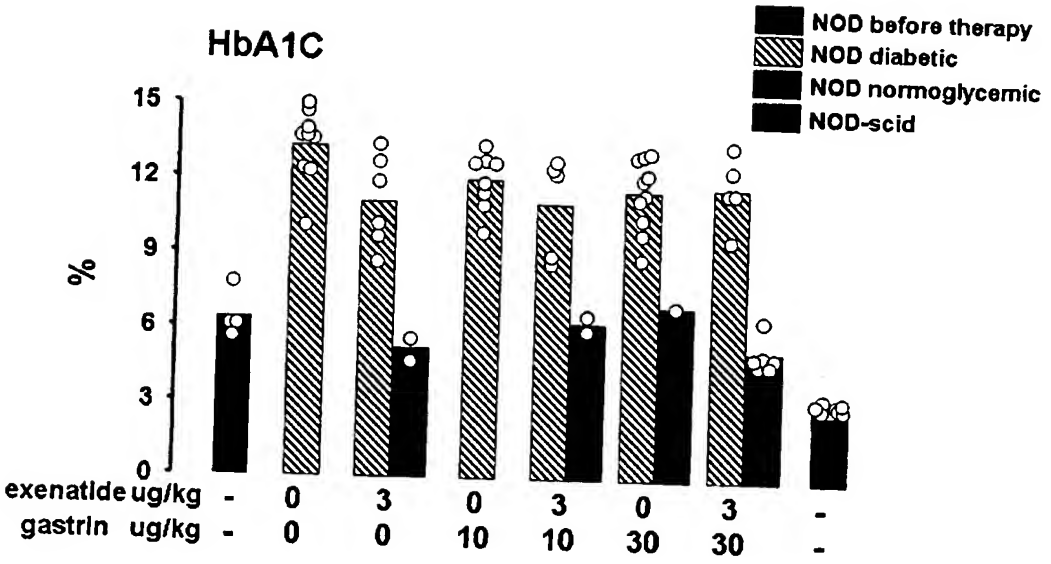

HbA1C
NOD before therapy
NOD diabetic
NOD normoglycemic
NOD-scid
%
15
12
9
6
3
0
exenatide ug/kg - 0 3 0 3 0 3 -
gastrin ug/kg - 0 0 10 10 30 30 -

Figure 5

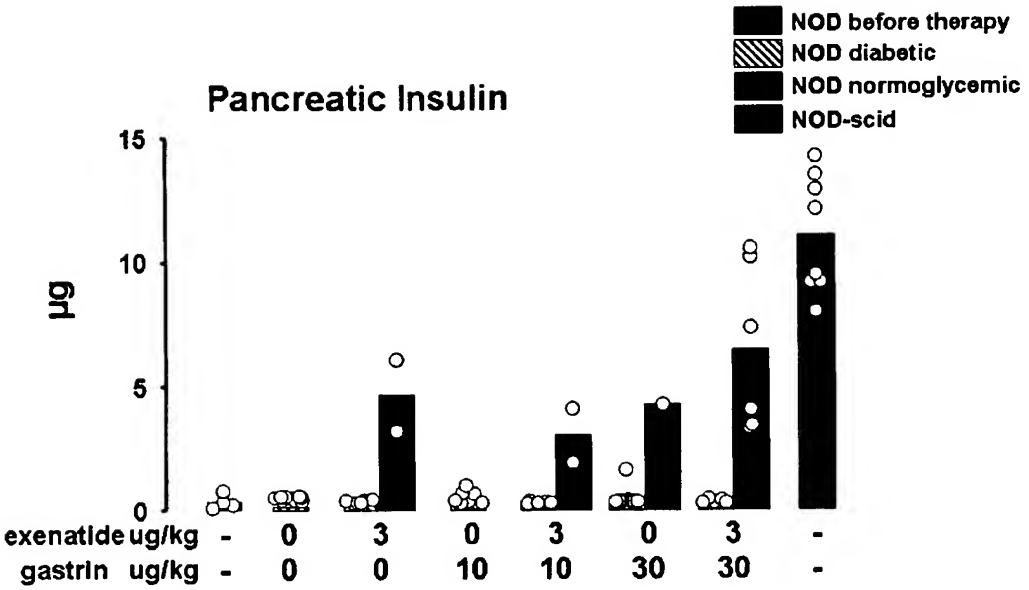

NOD before therapy
NOD diabetic
NOD normoglycemic
NOD-scid
Pancreatic Insulin
µg
15
10
5
0
exenatide ug/kg - 0 3 0 3 0 3 -
gastrin ug/kg - 0 0 10 10 30 30 -

Figure 6

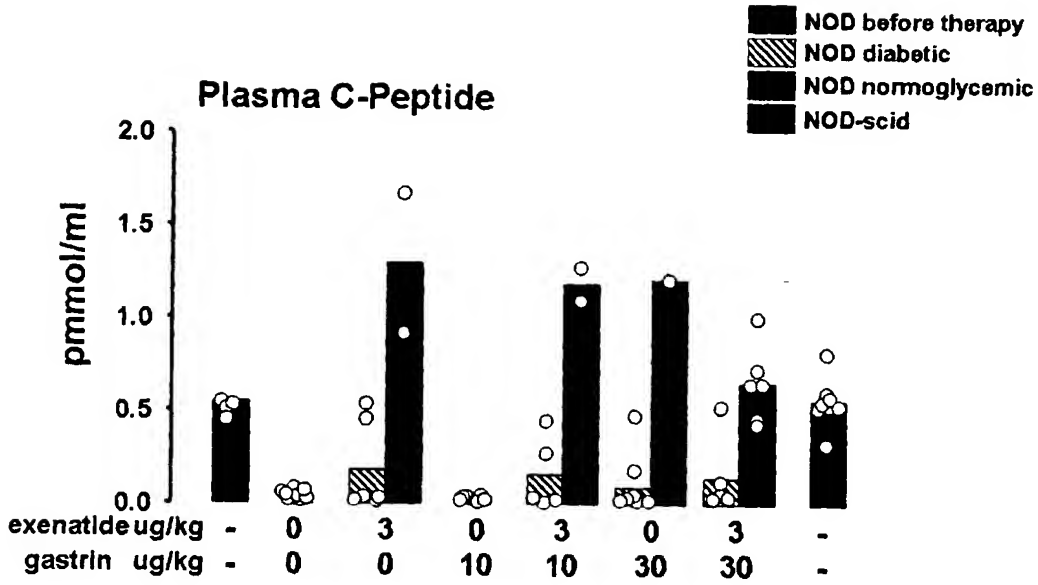

NOD before therapy
NOD diabetic
NOD normoglycemic
NOD-scid
Plasma C-Peptide
pmmol/ml
2.0
1.5
1.0
0.5
0.0
exenatide ug/kg - 0 3 0 3 0 3 -
gastrin ug/kg - 0 0 10 10 30 30 -

Figure 7

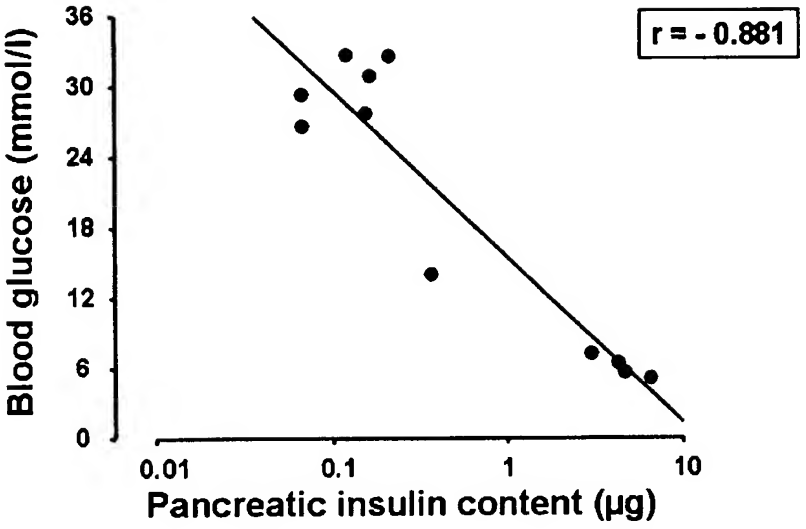

r = - 0.881
Blood glucose (mmol/l)
36
30
24
18
12
6
0
0.01
0.1
1
10
Pancreatic insulin content (µg)

Figure 8

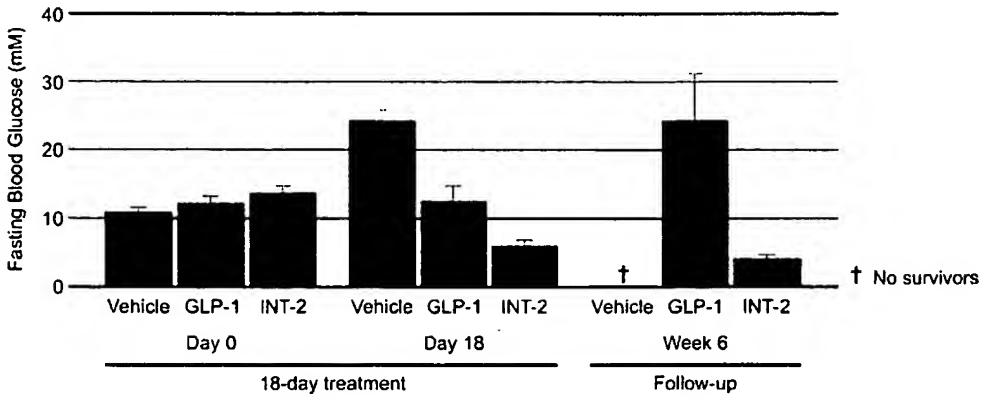

Fasting Blood Glucose (mM)
40
30
20
10
0
Vehicle
GLP-1
INT-2
Vehicle
GLP-1
INT-2
Vehicle
GLP-1
INT-2
†
† No survivors
Day 0
Day 18
Week 6
18-day treatment
Follow-up

Figure 9

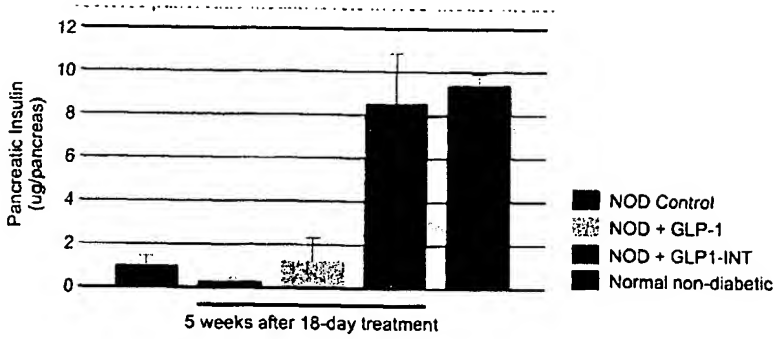
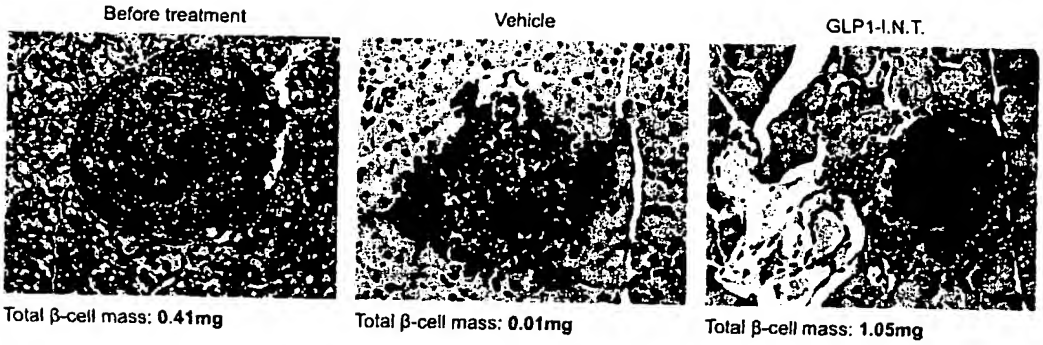

12
10
8
6
4
2
0
Pancreatic Insulin (ug/pancreas)
5 weeks after 18-day treatment
NOD Control
NOD + GLP-1
NOD + GLP1-INT
Normal non-diabetic

Figure 10


Before treatment
Vehicle
GLP1-I.N.T.
Total β-cell mass: 0.41mg
Total β-cell mass: 0.01mg
Total β-cell mass: 1.05mg

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/000266

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 19/00*** (2006.01), ***A61K 38/22*** (2006.01), ***A61P 3/10*** (2006.01), ***A61P 5/50*** (2006.01), ***C07K 14/595*** (2006.01), ***C07K 14/605*** (2006.01), ***C12N 5/02*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: C07K 19/00 (2006.01), A61K 38/22 (2006.01), A61P 3/10 (2006.01), A61P 5/50 (2006.01), C07K 14/595 (2006.01), C07K 14/605 (2006.01), C12N 5/02 (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
databases PubMed, Scopus, Delphion, Canadian Patent Database. Keywords: gastrin, exendin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2005072045 A2 (WARATAH PHARMACEUTICALS, INC.), 11 August 2005 (page 1, line 19 to 24; page 11, line 17 to 33; page 12 line 8 to page 15, line 28; page 16, line 27 to page 19, line 11, page 22, line 8 to 21 and seq ID 8)	1 - 35
X	WO2004037195 A2 (WARATAH PHARMACEUTICALS, INC.), 6 May 2004 (page 2, line 9 to 13; page 12, line 16 to line 33; page 17, line 21 to page 18, line 8; page 21 line 8 to line 19; page 44, example 1)	1 - 35

[ ] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 March 2007 (28-03-2007)	4 June 2007 (04-06-2007)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Antonio Candeliere 819- 934-7935

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/000266

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 21 - 32
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 21 - 32 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search.. Regardless, this Authority has established a written opinion based on the alleged effect or uses of the product defined in claims 21 - 32.

2. [ ] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2007/000266

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2004037195	06-05-2004	AU2003243501 A1	22-12-2003
		AU2003283004 A1	13-05-2004
		AU2003285229 A1	15-06-2004
		BR0315523 A	30-08-2005
		BR0316489 A	11-10-2005
		CA2486584 A1	18-12-2003
		CA2501677 A1	06-05-2004
		CA2505167 A1	03-06-2004
		CN1671407 A	21-09-2005
		CN1729016 A	01-02-2006
		CN1738644 A	22-02-2006
		EP1511509 A1	09-03-2005
		EP1565212 A1	24-08-2005
		EP1569680 A2	07-09-2005
		IL165285D D0	18-12-2005
WO2005072045	11-08-2005	AU2005207870 A1	11-08-2005
		CA2554458 A1	11-08-2005
		EP1711532 A2	18-10-2006